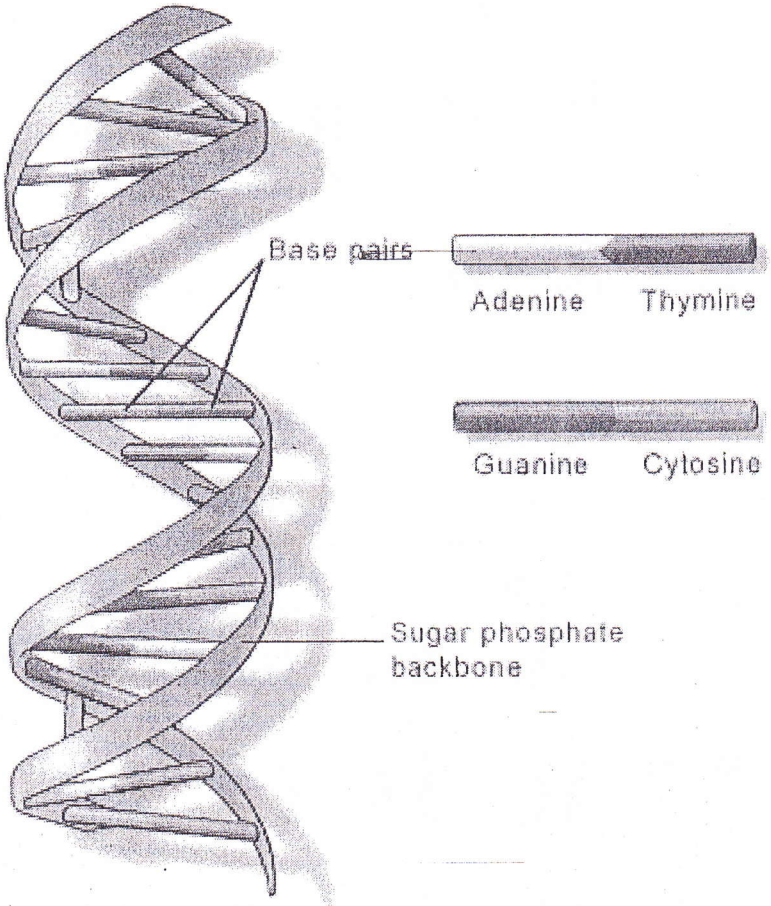


Medical Genetics

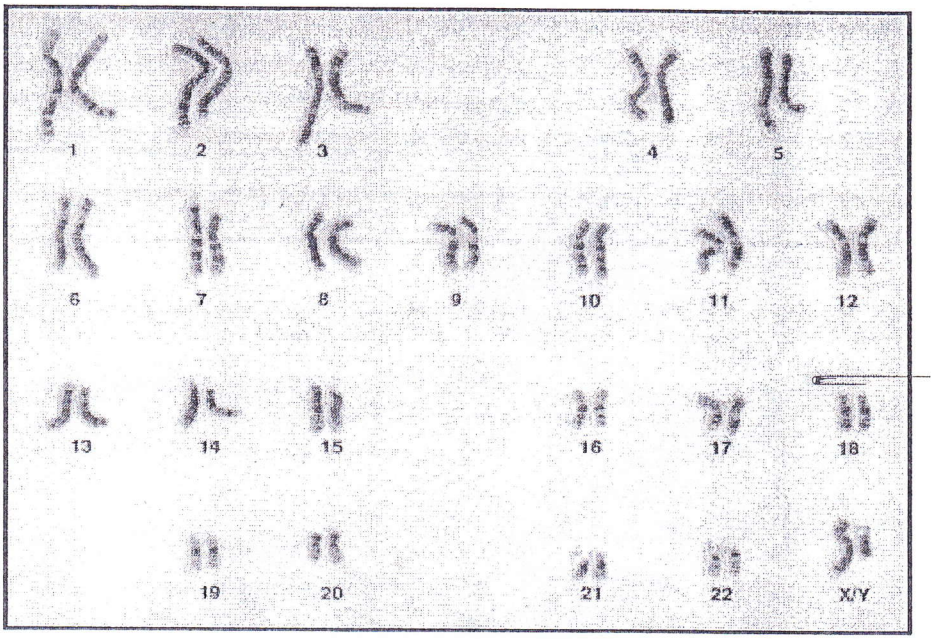
By

Ahmed M. Badr (MD)

Lecturer of Pediatrics

Cairo University

2010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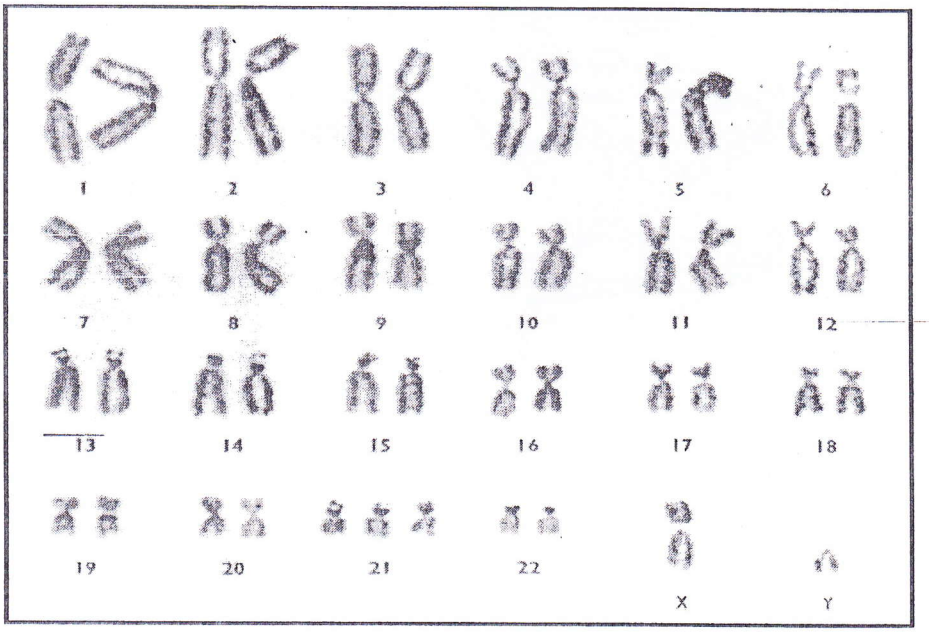
X/Y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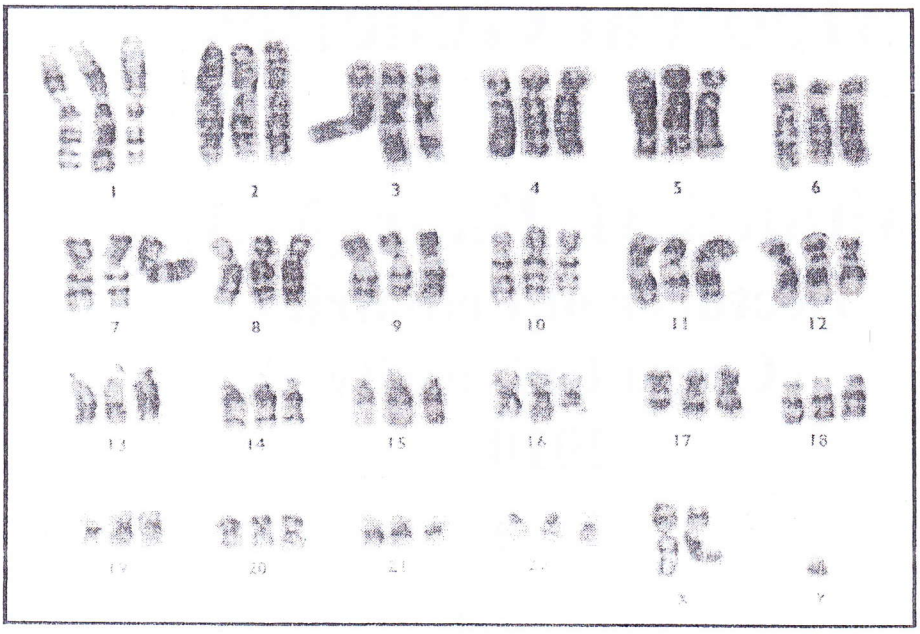
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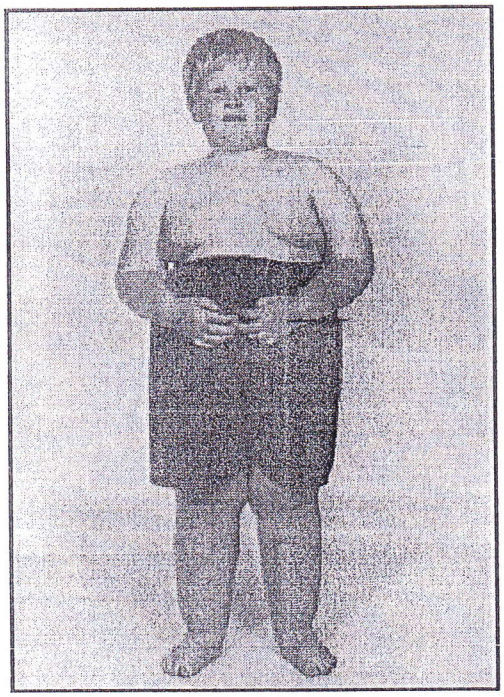
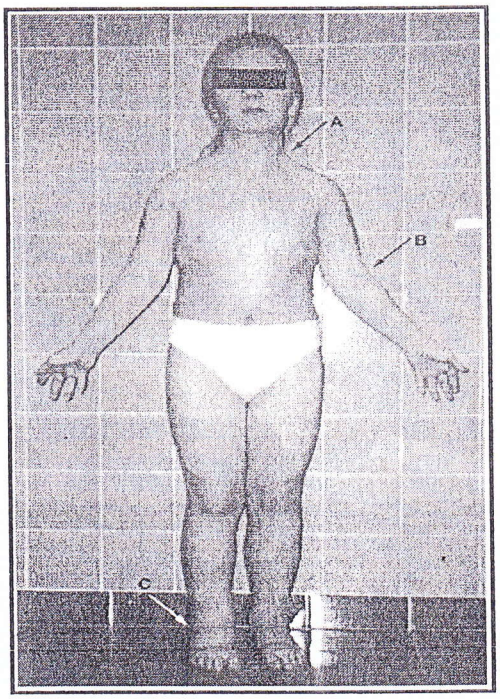
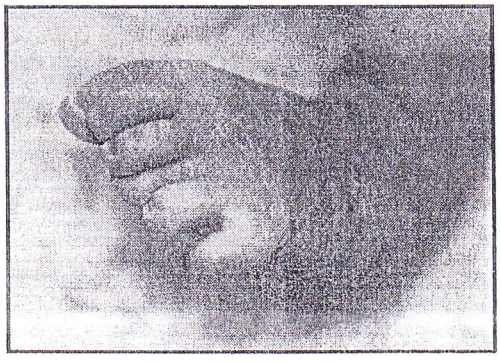
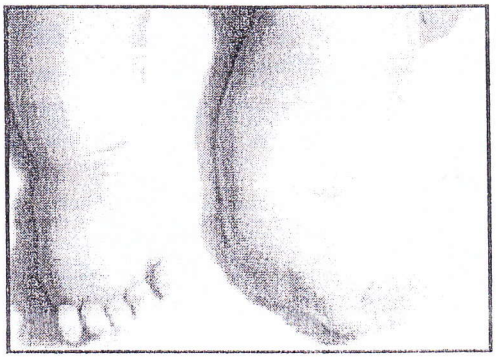
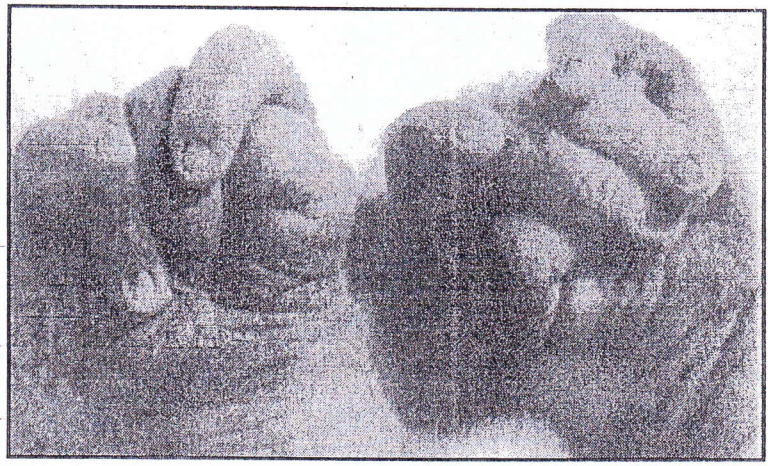
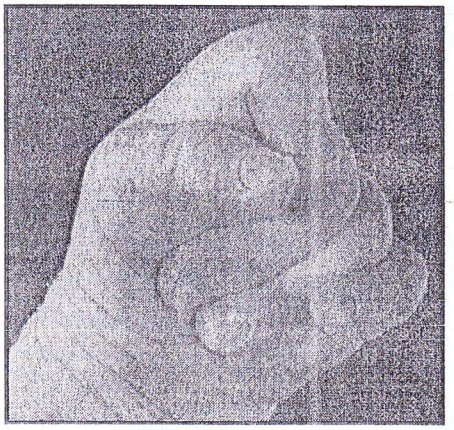


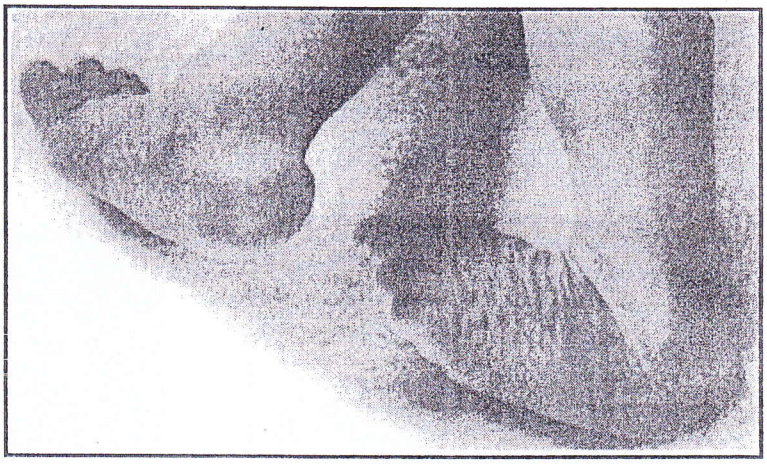

A
B
C

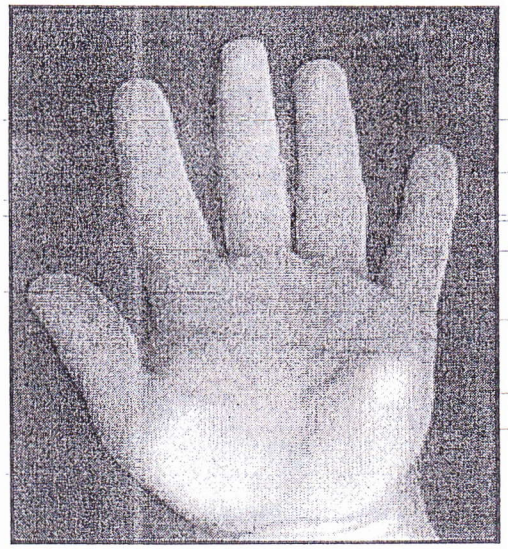


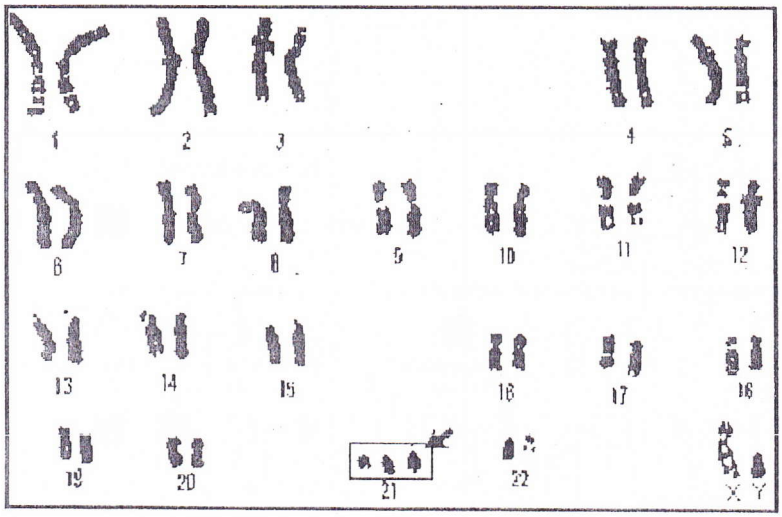


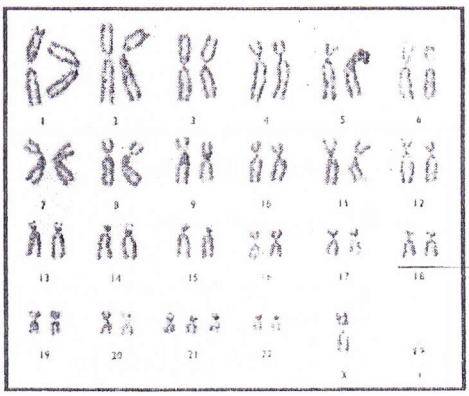


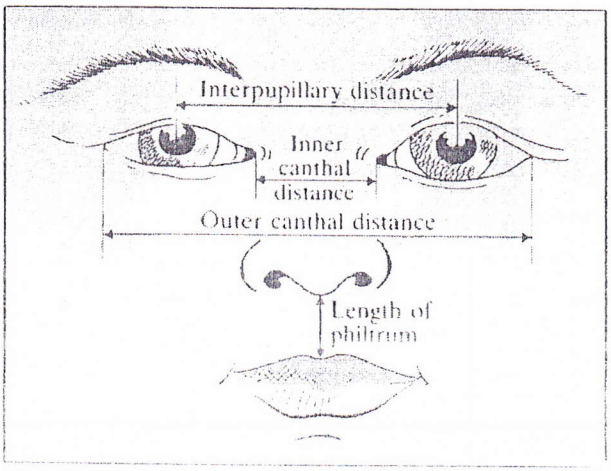


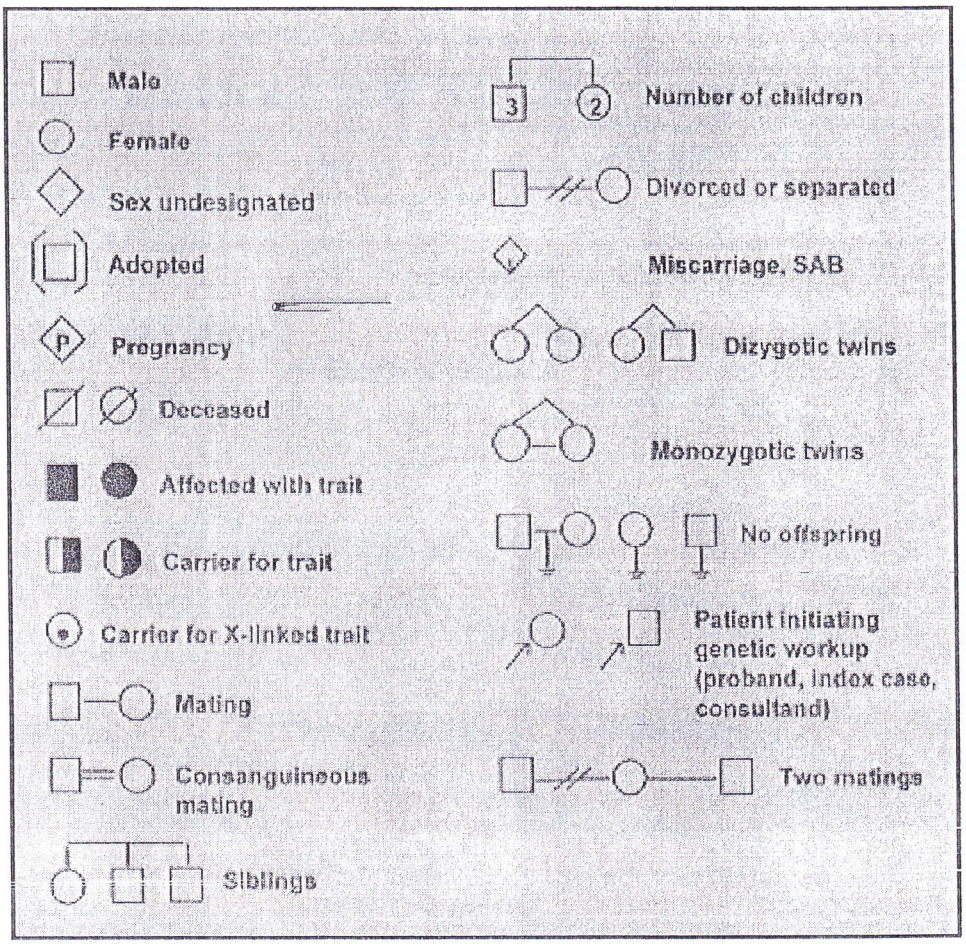


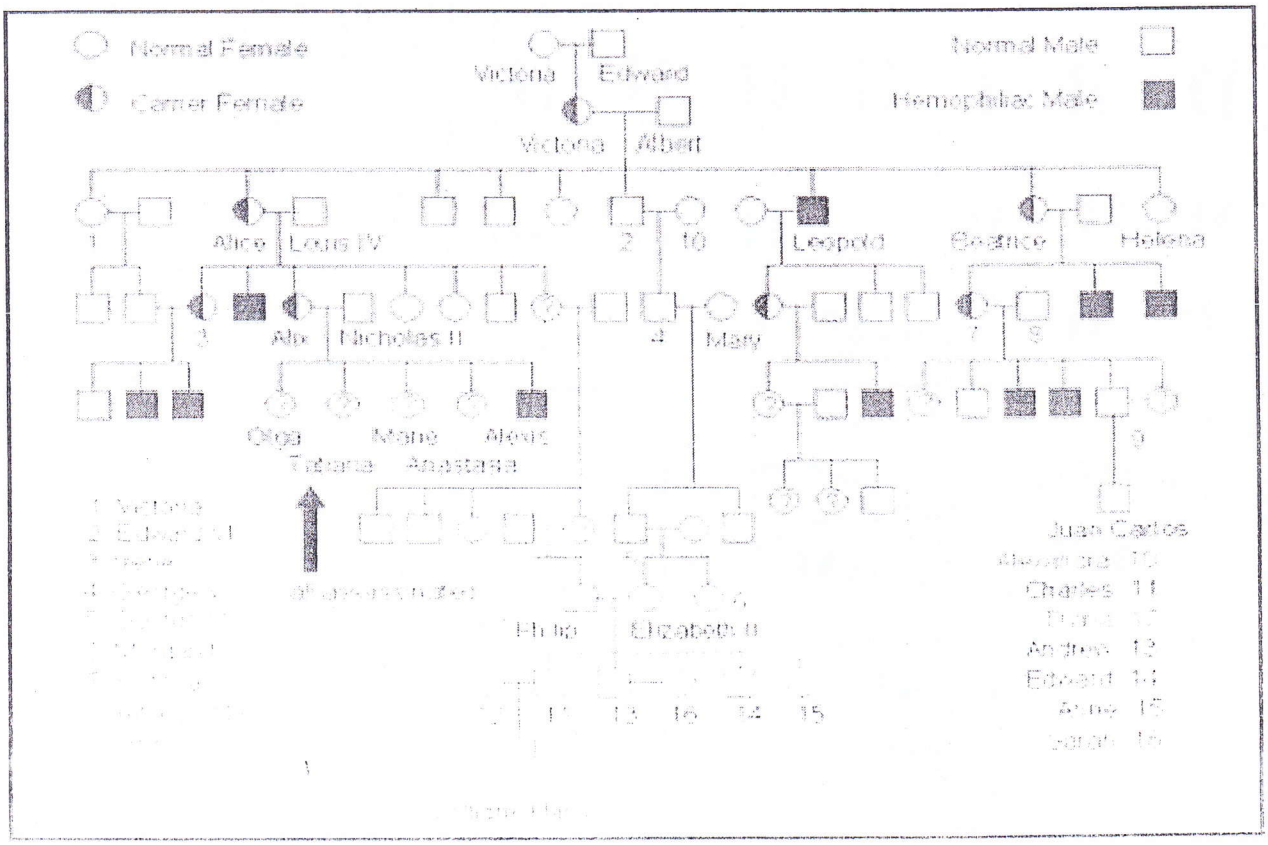


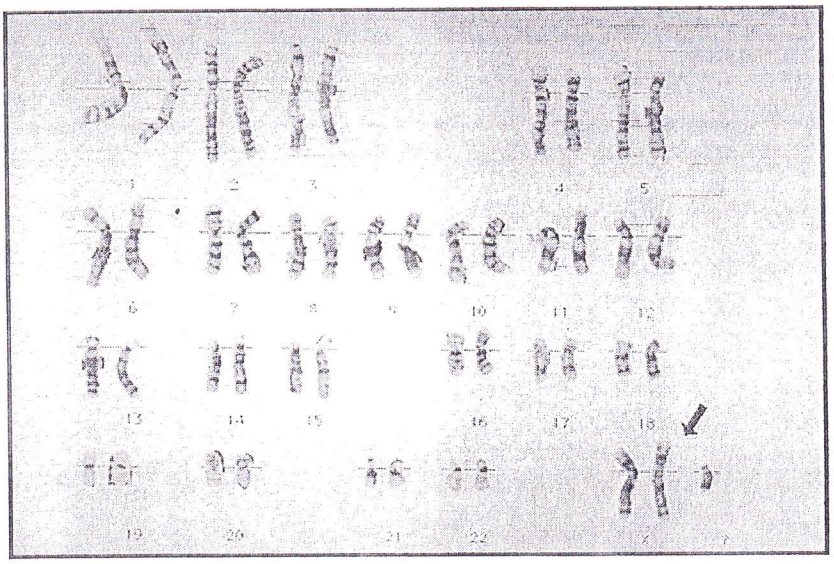


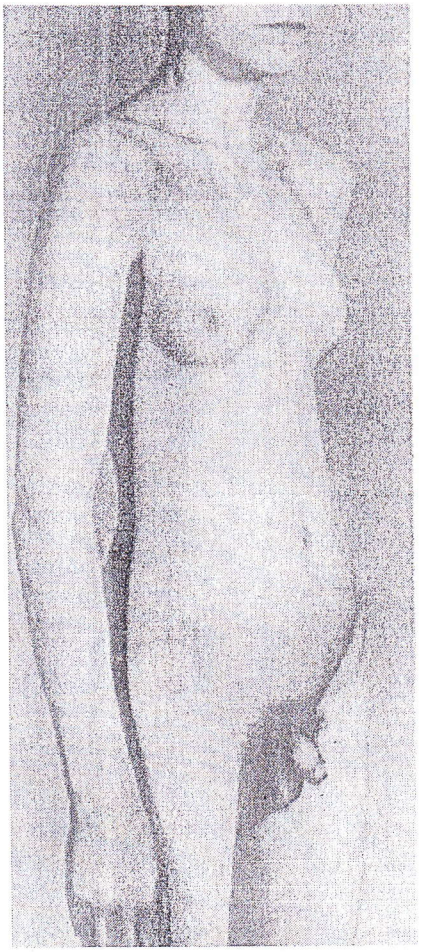


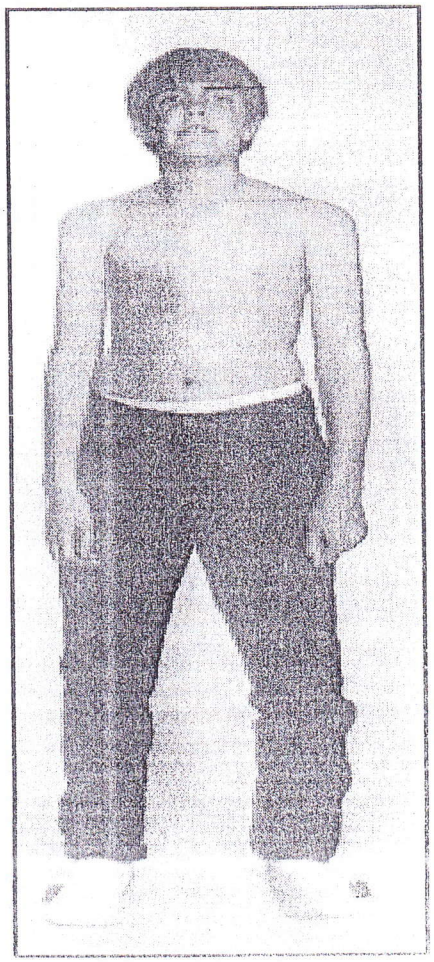

Interpupillary distance
Inner canthal distance
Outer canthal distance
Length of philtrum

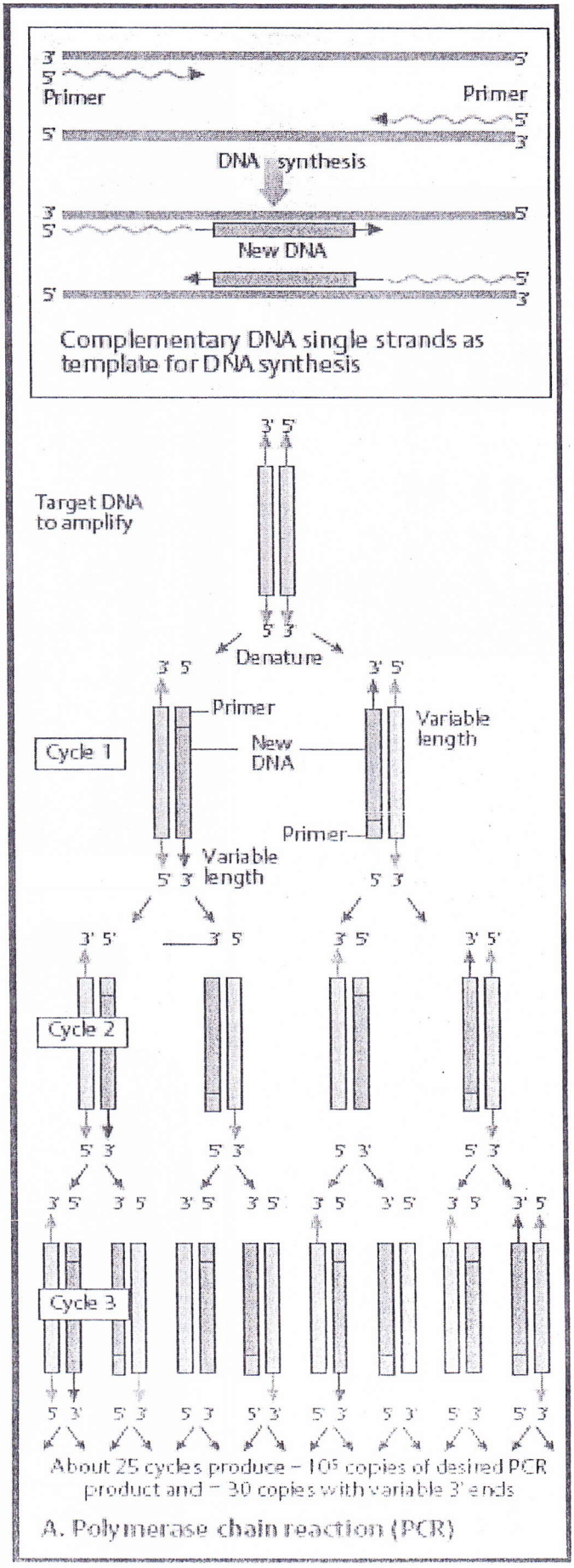

Male
Female
Sex undesignated
Adopted
P
Pregnancy
Deceased
Affected with trait
Carrier for trait
Carrier for X-linked trait
Mating
Consanguineous mating
Siblings
3
2
Number of children
Divorced or separated
Miscarriage, SAB
Dizygotic twins
Monozygotic twins
No offspring
Patient initiating genetic workup (proband, index case, consultand)
Two matings


Normal Female
Carrier Female
Victoria
Edward
Victoria
Albert
Normal Male
Hemophiliac Male
Alice
Louis IV
Leopold
Beatrice
Helena
Alix
Nicholas II
Olga
Tatiana
Maria
Anastasia
Alexei
Mary
Juan Carlos
Charles
Andrew
Edward
Anne
Hugo
Elizabeth II








3'
5'
Primer
Primer
5'
3'
DNA synthesis
New DNA
Complementary DNA single strands as template for DNA synthesis
Target DNA to amplify
Denature
Primer
New DNA
Variable length
Cycle 1
Primer
Variable length
Cycle 2
Cycle 3
About 25 cycles produce ~ 10^6 copies of desired PCR product and ~ 30 copies with variable 3' ends
A. Polymerase chain reaction (PCR)

Cell Structure

The cell is formed of:

A) Nucleus:

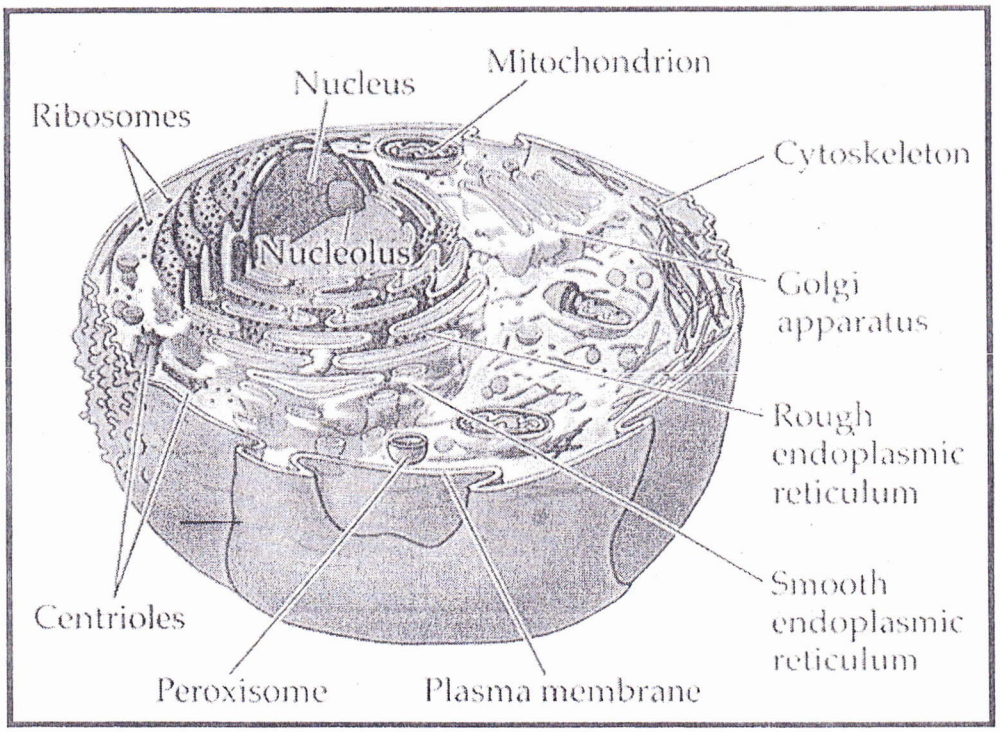
- ☒ Nuclear membrane
- ☒ Nucleolus
- ☒ Nuclear matrix
- ☒ Chromatin: During cell division, chromatin is *condensed* into separate chromosomes

Remember
All cells have nuclei except RBCs

B) Cytoplasm: Contains the following organelles

- ☒ Endoplasmic reticulum *"Transport"*
- ☒ Golgi apparatus *"Protein synthesis"*
- ☒ Mitochondria *"Energy production"*
- ☒ Ribosomes *"Protein synthesis"*
- ☒ Peroxisomes *"Fatty acid metabolism"*

The total length of all DNA strands = 2 meters



Human Chromosomes

Definition

Thread-like structures found in the nucleus and formed of:

a. **DNA** (Deoxyribonucleic acid): carries the genetic information (Genes)
b. **Proteins** [Histones & non-histones]: responsible for DNA coiling (Packing)

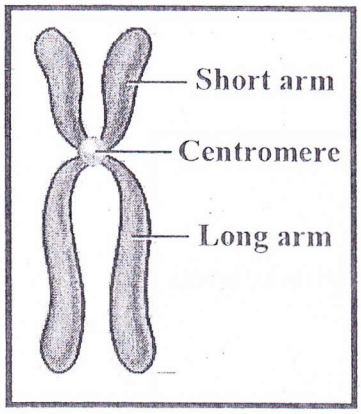
Number

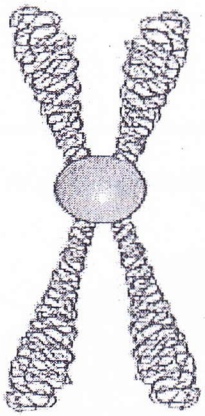
- Each **somatic** cell contains 46 chromosomes (23 pairs = 2 n = diploid), classified into:
  a. 22 pairs of homologous (similar) chromosomes called **autosomes**
  b. One pair of **sex chromosomes**: XX in ♀ & XY in ♂
- Each **gamete** (germ cells: ovum & sperm) contains 23 chromosomes (23 = n = haploid)
  a. 22 autosomes
  b. One sex chromosomes: X in ova & X or Y in sperms
- The **zygote** contains 46 chromosomes: 23 chromosomes from each parent

Structure of Chromosomes

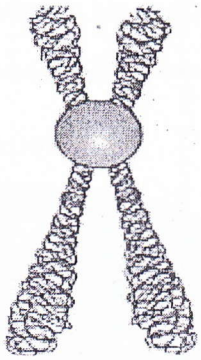
- During cell division, each chromosome is formed of 2 chromatids connected together at the centromere. The centromere divides the chromosome into:
  - **a.** Short arm = p arm (**p** for **p**etit)
  - **b.** Long arm = q arm
- Chromosomes can be classified according to position of the centromere into:
  - ➢ Metacentric: Centromere is very near to the center
  - ➢ Submetacentric: Centromere midway between the center & end
  - ➢ Acrocentric: Centromere is very near to the end
- Chromosomal ends are called **telomeres**

NB:Anti-centromere Ab in scleroderma

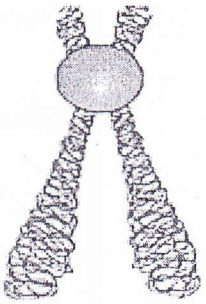




Metacentric



Submetacentric



Acrocentric

Gene

- It is a DNA sequence that directs the synthesis of a specific polypeptide chain
- Number of human genes is about 25.000 genes

Locus

It is the **site** of a gene on a chromosome

Allele

- It is alternative form of a gene found at the same locus on a chromosome. Alleles on homologous chromosomes may be:
- Each trait is controlled by 2 alleles (One from each parent), which may be:
  - **a. Homozygous:** Identical (they may be normal or not)
  - **b. Heterozygous:** Different
  - **c. Hemizygous:** there is only one set for genes on X or Y chromosomes in **males**

Homologous chromosomes

- They are chromosomes which pair during meiosis & contain identical loci
- X & Y chromosomes are **not** homologous (Different in size & shape)

Dominant gene

It expresses itself whether homozygous or heterozygous

Recessive gene

It expresses itself only if homozygous

Codominant genes

Both genes are expressed in the heterozygous

Cell Cycle

Definition

- It is the time taken by the cell to divide into 2 daughter cells.
- It is divided into interphase (3 stages) & mitosis:

Stage		Duration	Events
Interphase	G1 (gap 1)	9 hours	Formation of enzymes & nucleotides
	S (synthesis)	9 hours	DNA synthesis (gene duplication)
	G2 (gap 2)	4 hours	Preparation for mitosis
Mitosis		1-2 hours	Cell division

G0 (gap 0) is the stage of quiescent cells which may be transient or permanent (nerve cells)

Cell Division

There are 2 types of cell division:

a. Mitosis: occurs in somatic cells ⟶ 2 daughter cells (2 n) "Diploid number"

b. Meiosis: occurs in germ cells ⟶ 4 daughter cells (n) "Haploid number"

- ☒ First meiotic division: Reduction division
- ☒ Second meiotic division: as mitosis

Mitosis	Meiosis (1st meiotic division)
Preceded by Interphase	
A) Prophase: • Nucleus: swell • Nuclear membrane: disappears • Chromatin: **condensation** into chromosomes • Chromosomes: visible; formed of 2 chromatids • Centrioles: migrate to the opposite poles • Spindle: formed & attached to chromosomes	**A) Prophase I:** • **Leptotene** *(thread)*: chromosomes are thread-like • **Zygotene** *(pairs)*: chromosomes are paired • **Pachytene** *(thick)*: chromosomes are formed of chromatids + crossing over (2 chromatids) • **Diplotene** *(double)*: tetrad + chiasmata • **Diakinesis** *(motion)*: homologous chromosomes start to move apart
B) Metaphase Chromosomes are **arranged** in the equatorial plane *(Homologous chromosomes do **not** react)*	**B) Metaphase I** Homologous chromosomes are arranged in the equatorial plane
C) Anaphase • **Contraction** of the spindle • Separation of sister chromatids at the centromere • Each chromatid is now called chromosome	**C) Anaphase I** • Contraction of the spindle • Separation of homologous chromosomes
D) Telophase • **Cleavage** of the cytoplasm & cell membrane • Decondensation of chromosomes ⟶ chromatin • 2 daughter cells (2 n) are formed "diploid"	**D) Telophase I** • Cleavage of the cytoplasm & cell membrane • Decondensation of chromosomes ⟶ chromatin • 2 daughter cells (n) are formed "haploid"
Meiosis (2nd meiotic division) = As mitosis (pro-, meta-, ana- & telophase)	

Crossing over (recombination):

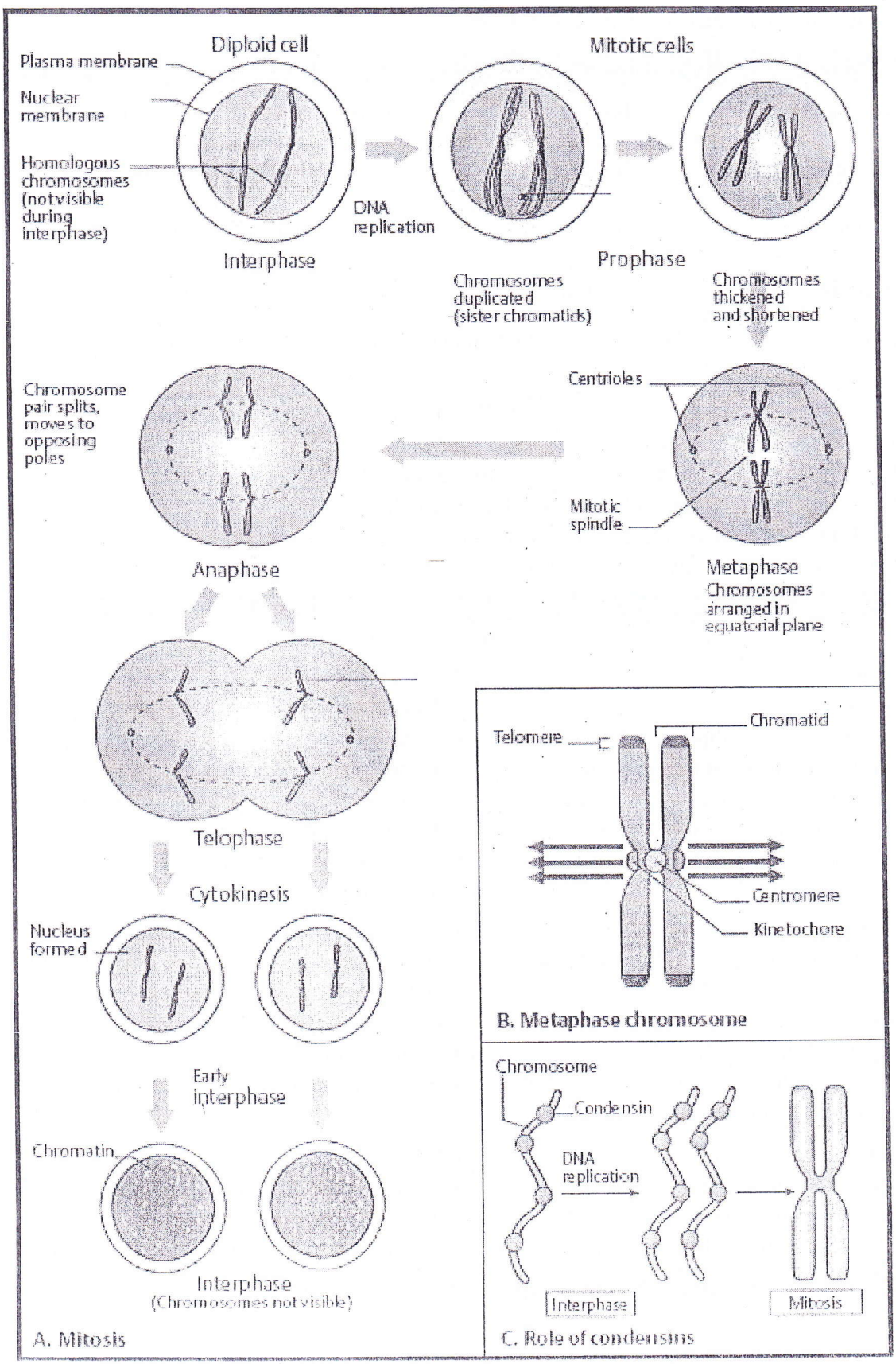
Definition: Physiologic process occurring during **meiosis** (Pachy- & Diplotene) at the area of chismata

Process: Breakage & exchange of genetic material between chromatids of *homologous* chromosomes

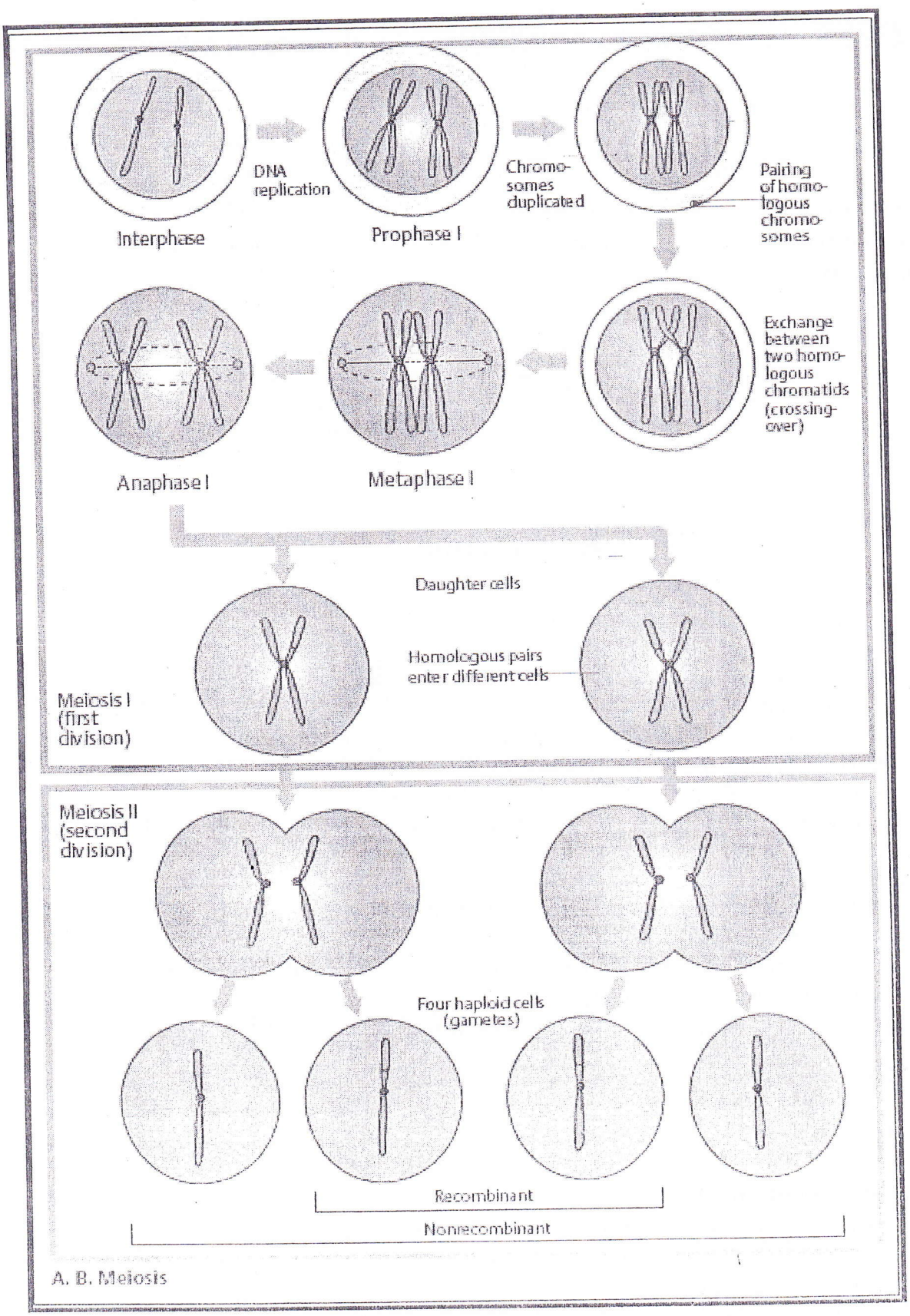
Value: Genetic recombination (variation)

Crossing over between 2 **sister** chromatids occurs in Bloom syndrome (↑ Risk of malignancy)

Mitosis


Diploid cell
Plasma membrane
Nuclear membrane
Homologous chromosomes (not visible during interphase)
Interphase
DNA replication
Mitotic cells
Prophase
Chromosomes duplicated (sister chromatids)
Chromosomes thickened and shortened
Centrioles
Mitotic spindle
Metaphase
Chromosomes arranged in equatorial plane
Chromosome pair splits, moves to opposing poles
Anaphase
Telophase
Cytokinesis
Nucleus formed
Early interphase
Chromatin
Interphase
(Chromosomes not visible)
A. Mitosis
Telomere
Chromatid
Centromere
Kinetochore
B. Metaphase chromosome
Chromosome
Condensin
DNA replication
Interphase
Mitosis
C. Role of condensins

Meiosis



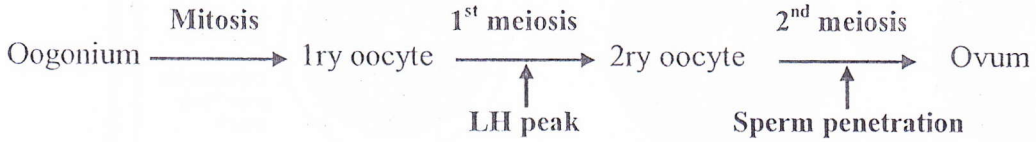
A. B. Meiosis

Gametogenesis

Definition

It is the production of gametes (sperm or ovum); by spermatogenesis or oogenesis

	Spermatogenesis	Oogenesis
Site	Seminiferous tubules	Ovary
Onset	At puberty	Fetal life
Course	Continuous	Interrupted at Prophase I & Metaphase II
Mitosis	Spermatogonia ⟶ 1ry spermatocyte	Oogonia ⟶ 1ry oocyte
1st meiotic division	2 secondary spermatocytes	One 2ry oocyte + 1 polar body
2nd meiotic division	4 sperms	One ovum + 3 polar bodies
Telophase I, II	Equal division of the cytoplasm	Unequal division of the cytoplasm



Karyotyping

Definition

It is the number, size & shape of the chromosomes in the cell

Methodology of Karyotyping

Chromosomes are best seen during metaphase

1. Type of the **cells**:
   - ☒ To study mitosis: lymphocytes, skin fibroblasts, amniocytes, chorionic villi cells
   - ☒ To study meiosis: Gonadal biopsy
2. **Culture** of the cells on suitable medium (to ↑↑ number)
3. Stimulation of cell division by adding **mitogenic** agent [Phytohemagglutinin (PHA)]
4. **Incubation** for 72 hrs
5. Inhibition of cell division at **metaphase** by colchicine (It inhibits spindle formation)
6. Addition of **hypotonic** solution ⟶ Cell swelling & rupture

   As in osmotic fragility test
7. Separation & **staining** of chromosomes
   - ☒ G banding: Staining with Giemsa stain ⟶ alternating dark & light bands
   - ☒ Q fluorescence using quinacrine
8. Chromosomes are photographed, cut & **arranged** in pairs in a standard manner

Number
Structure } See before

Classification of chromosomes

A) **According to the shape (position of the centromere):**

1. Metacentric: Centromere is very near to the center
2. Submetacentric: Centromere midway between the center & end
3. Acrocentric: Centromere is very near to the end
4. Satellite: a small part of chromatin is attached by a narrow stalk to the short arm of acrocentric chromosome

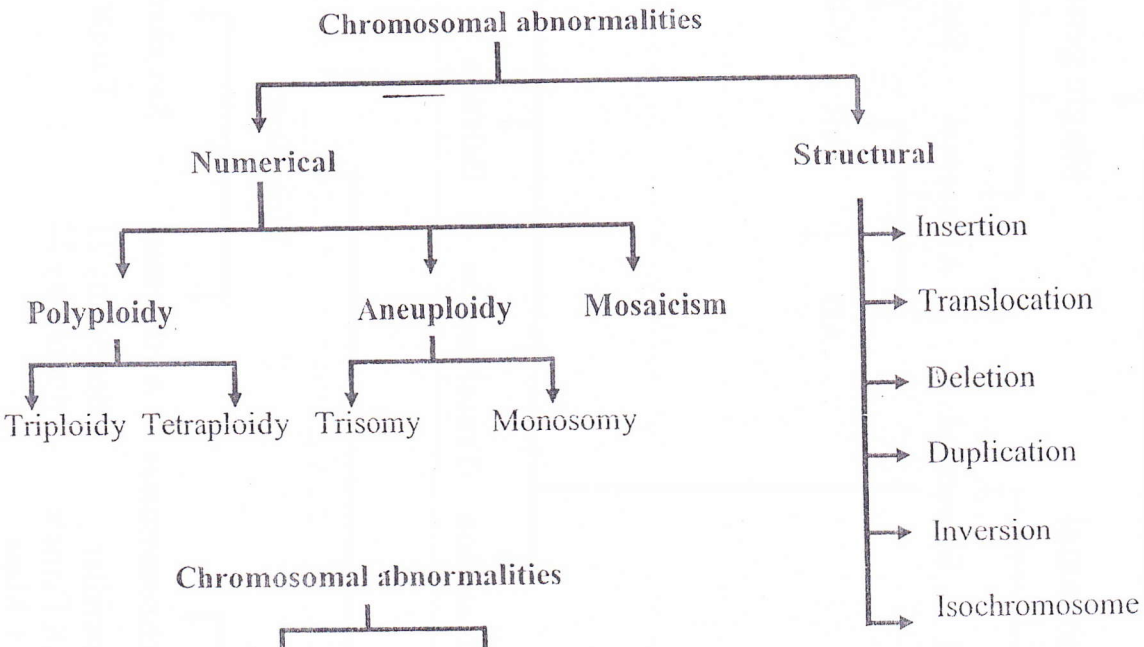
B) According to the size:

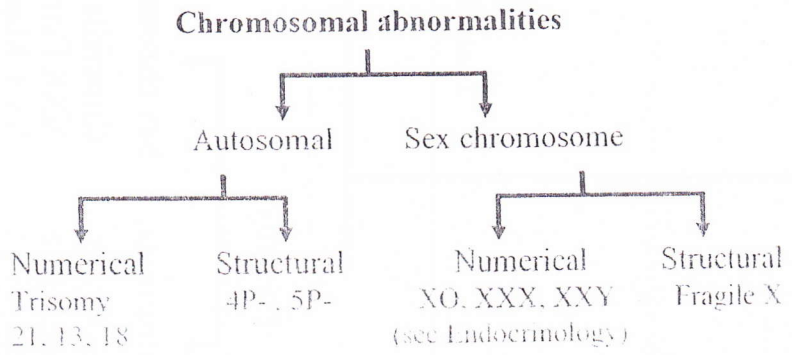
- ☒ Group A: 1-3
- ☒ Group B: 4,5
- ☒ Group C: 6-12 & X
- ☒ **Group D:** 13-15
- ☒ Group E: 16-18
- ☒ Group F: 19,20
- ☒ **Group G:** 21,22 & Y

The largest is 1
The smallest is 21??

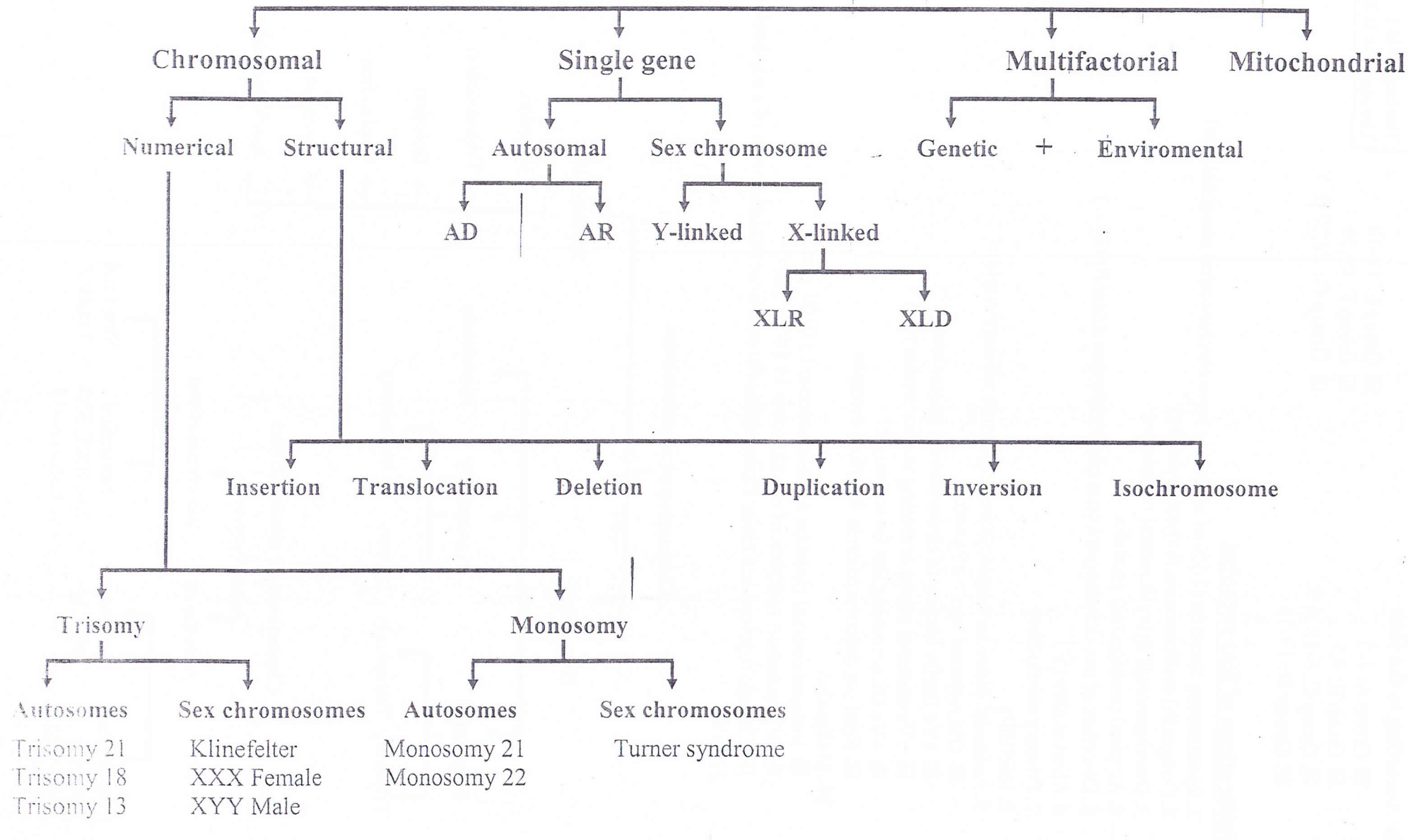
Indications of Karyotyping

1. Spontaneous abortion (7.5% of abortions have chromosomal abnormalities)
2. Congenital malformation & dysmorphology
3. Developmental delay & mental retardation
4. Atypical (ambiguous) genitalia
5. Disorders of sex development (complete androgen insensitivity…)
6. Short stature (♀)
7. Primary amenorrhea
8. Infertility
9. Antenatal (done on amniocytes or chorionic villous sample):
   - ☒ Old maternal age > 35 years
   - ☒ +Ve family history of chromosomal anomalies
   - ☒ +Ve maternal serum screening test for trisomy 21
   - ☒ +Ve US screening test for trisomy 21
   - ☒ Fetal sex determination in X-linked diseases
10. Malignancy
    - ☒ Retinoblastoma: mutation of chromosome 13 (RB1 gene)
    - ☒ Wilms tumor: mutation of chromosome 11 (WT1 gene)
    - ☒ Chronic myeloid leukemia: Philadelphia chromosome (translocation of a segment of 22 to 9)





Genetic Disorders



Chromosomal abnormalities

Definition

Abnormalities in the number or the structure of the chromosomes

Classification

A) **Numerical aberrations:**

1. **Polyploidy:**

Any multiple of the haploid number of chromosomes *(except normal diploid)*

a. Triploidy [3 n = 3 x 23 = 69 chromosomes]

Causes:

- ☒ Failure of spindle formation during 1st *meiotic* division (in gametogenesis)
- ☒ Fertilization of 1 ovum with 2 sperms

b. Tetraploidy [4 n = 4 x 23 = 92 chromosomes]

Causes:

- ☒ Failure of telophase of the 1st *mitotic* division of the zygote

2. **Aneuploidy**

Abnormal number of a particular chromosome *(normally each chromosome has 2copies)*

a. Trisomy

Definition: One chromosome is represented by 3 copies not 2 "extra chromosome"

Examples: Trisomy 21, 18, 13

b. Monosomy

Definition: One chromosome is represented by one copy not 2 "absent chromosome"

Examples: Turner syndrome (XO)

Causes of aneuploidy:

- ☒ Non-disjunction during meiosis:
  - Anaphase I: (non-disjunction of homologous chromosomes)
  - Anaphase II: (non-disjunction of sister chromatids)
- ☒ Anaphase lag: (Delayed movement of a chromosome during anaphase)

Causes of non-disjunction:

- ☒ Old maternal age (↑↑ Teratogen exposure, weak chiasmata)
- ☒ Ionizing & non ionizing radiation
- ☒ Chemicals e.g., alkylating agents
- ☒ Familial tendency

3. **Mosaicism & chimerism**

Mosaicism: the presence of 2 or more different cell lines derived from 1 fertilized ovum

It is post-fertilization event

Chimerism: the presence of 2 or more different cell lines derived from >1 fertilized ovum

Examples: twins (TTTS), bone marrow transplantation, recombinant DNA

B) **Structural aberrations:**

Etiology:

- ☒ Ionizing & non-ionizing radiation
- ☒ Chemicals e.g., alkylating agents
- ☒ Inherited diseases
- ☒ Spontaneous

Inherited causes of chromosomal breakage:

1. Fanconi anemia
2. Ataxia telangiectasia
3. Bloom syndrome
4. Xeroderma pigmentosa

Some changes occur in the genome through natural processes e.g., crossing-over during meiosis

1. Insertion *"ins"*

One piece of chromosome breaks at 2 points & is incorporated into a break in the same or other chromosome (3 breaks are needed)

2. Translocation

a. Reciprocal translocation *"t"*

Exchange of genetic material between non-homologous chromosomes

Usually it does not cause any abnormality (except if disruption of a normal gene occurs)

b. Robertsonian translocation *"rob"*

- Deletion of the short arms of 2 acrocentric chromosomes & centromere of one of them
- The long arm& centromere of one chromosome is translocated to the long arm of the other
- So, the total number of chromosome is 45 (numerical aberration)
- But with *normal* phenotype **[Balanced translocation carrier]**

3. Deletion *"del"*

Loss of part of chromosome

a. Terminal

Loss of one end of the chromosome (one break)

b. Interstitial

Loss of part between 2 fragments of the chromosome (2 breaks)

c. Ring

Loss of both ends of the chromosome (2 breaks), with folding "ring formation"

Deletion may be detected by routine chromosomal preparations (5p = Cri-du-chat)
Microdeletions can be detected by cytogenetic studies (FISH)

Microdeletion Syndromes

Syndrome	Chromosome
1. Prader-Willi	15
2. Angelman	15
3. Williams	7
4. WAGR	11
5. Alagille	20
6. DiGeorge	22

4. Duplication *"dup"*

The presence of 2 copies of a segment of a chromosome
It results from unequal crossing-over during meiosis
Example: Charcot-Marie-Tooth diasease (HMSN-I)

5. Inversion *"inv"*

End-to-end reversal of a segment within a chromosome (2 breaks are needed)

- Pericentric: around the centromere (both arms)
- Paracentric: involving one arm

6. Isochromosome *"iso"*

Transverse division of a chromosome at the centromere during meiosis followed by duplication → One chromosome with 2 long arms
One chromosome with 2 short arms

Down syndrome

(Trisomy 21)

Definition

It is numerical chromosomal abnormality (Trisomy 21) in which the cell contains 3 copies of chromosome number 21 instead of 2 (i.e., Extra chromosome 21)

Incidence 1: 700

Genetic Types

- ☒ Non-disjunction (95%): Usually during maternal meiosis [Resulting in ovum with 24 chromosomes (Two chromosome 21 instead of one)]
- ☒ Translocation (4%): The extra-21 chromosome is translocated to another acrocentric chromosome (group **D** 13-15 or group **G** 21-22)
  One of the parents should be translocation carrier **[= Balanced translocation carrier]**
- ☒ Mosaicism (1%): Post-fertilization event [= Non-disjunction during zygote mitosis]

Number of chromosomes & Recurrence risk

Type	%	Mechanism	No of chromosome	Maternal age	IQ	Recurrence risk
Non-disjunction	95%	Non-disjunction 'Maternal meiosis'	47	Age dependent	Low	
Translocation	4%	Translocation	46 (The extra chromosome is translocated to another one)	Non-age dependent	Low	
Mosaicism	1%	Post-fertilization event	46/47	± Age dependent	~~Better~~	??

Recurrence risk of Down syndrome

A) Non-disjunction:

- Depends on the maternal age
- Risk is increased with advanced maternal age
- Examples:
  - At the age 20 yr = 1/2000
  - At the age 30 yr = 1/1000
  - At the age 40 yr = 1/100
  - At the age 50 yr = 1/10

B) Translocation: Depend on the chromosome to which the "extra-21" is translocated

a. Translocation to D-group
   1/3 Down
   1/3 normal
   1/3 translocation carrier

b. Translocation to chromosome 21
   100 % Down

C) Mosaicism
   Minimal

Clinical Picture

A) Delayed mental development (MR): Social smile, recognition of mother, speech …

B) Delayed motor development (hypotonia): Head support, sitting, crawling, walking…

C) Characteristic dysmorphic features:

☒ **Skull**
Brachycephaly, delayed closure of AF, microcephaly

☒ **Hair**
Silky

☒ **Eye**
Upward slanting palpebral fissure
Medial epicanthal folds
Speckled iris (brushfield iris)
Cataract (3 %), squint

☒ **Nose**
Depressed nasal bridge

☒ **Ears**
Malformed, overfolded helix
Underdeveloped ear lobule

☒ **Mouth**
Small oral cavity
Protruded & fissured Tongue

Remember
- **Down:** Small oral cavity
- **Cretinism:** Large tongue

☒ **Neck**
Short & broad
Atlantoaxial instability

☒ **Hands**
Short & broad hands
Clinodactyly (Incurved little finger)
Simian crease (Single transverse palmer crease)

50% of Down $ have simian crease
4% of population have simian cease

☒ **Feet**
Short & broad feet
Wide gap between 1st & 2nd toes (Sandal gap 97%)

☒ **Abdomen**
Distension, hernia

☒ **Chest**
Recurrent chest infections, why? ⇨

Causes of chest infection:
1. CHD
2. Hypotonia
3. Leukemia (↓↓Immunity)

☒ **Neurological**
Hypotonia
Alzheimer disease in the majority by the age of 40 yrs

D) Associated congenital anomalies:
- **a. Cardiac (40%):** Endocardial cushion defects (AV canal), VSD, ASD, PDA or Fallot
- **b. GIT (6%):** Duodenal atresia, annular pancreas, imperforate anus
- **c. Renal anomalies**

E) Complications:
- a. Cardiac complications (Heart failure)
- b. Recurrent chest infection
- c. Increased risk of leukemia (AML, ALL) 10-20 folds more than the general population
- d. Normal survival is expected in absence of complications (CHD)
- e. ↑↑ Risk of DM, obesity, thyroiditis & epilepsy

Investigations

A) Cytogenetic studies

- Karyotyping is essential in every patient to determine the type & recurrence risk
- In translocation Down, karyotyping of the parents and other relatives is required
- FISH

B) Imaging

- CXR, ECG, Echocardiography (CHD)
- Abdominal X-rays (Imperforate anus)
- Abdominal US (GIT & Renal anomalies)

Antenatal Diagnosis

Indications:

- ☒ Old maternal age > 35 years
- ☒ Previous baby with Down syndrome
- ☒ Family history of Down syndrome
- ☒ Family history of translocation

Methods:

1. Triple test: done in maternal serum at 15-16 weeks of gestation
   - ↓↓ α-Fetoprotein
   - ↓↓ Unconjugated estriol
   - ↑↑ β-hCG (Human chorionic gonadotropin)
2. Dimeric inhibin; marker in maternal serum (↑↑ in Down syndrome)
3. Fetal karyotyping:
   - Amniocentesis: 14-16 weeks of gestation
   - Chorionic villus sample: 9-12 weeks of gestation
   - Fetal cells in maternal circulation
4. Fetal US
   - Nuchal Translucency thickening: thickening of the nuchal fold at the back of the neck due to delayed drainage of fluid from the upper part of the body
   - Short femur
   - Cystic hygroma of the neck, duodenal stenosis

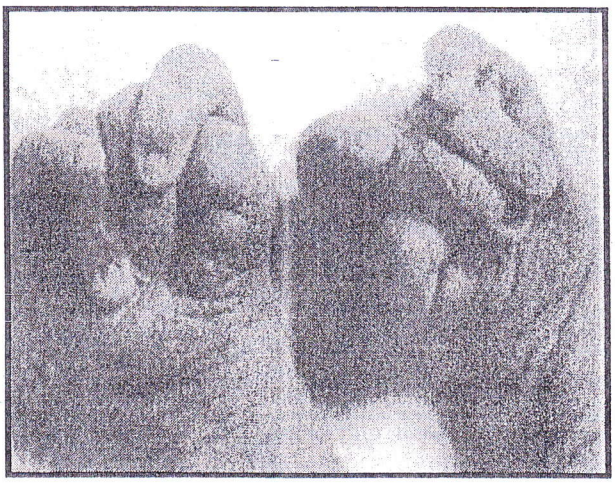
Do not say "a Down baby" but "a baby with Down syndrome"

Treatment

No specific Rx-Supportive
Rrehabilitation
Surgical correction of congenital anomalies

Common Autosomal Trisomies

	Trisomy 18 *(Edwards $)*	Trisomy 13 *(Patau $)*
Incidence	1/4000	1/6000
Genetic type	Non-disjunction	Non-disjunction
Features	Prominent occiput (Dolicho-) Microcephaly Eye anomalies Small ears Cardiac (VSD, PDA) Overlapping fingers (closed fist) Rocker-bottom (protruding calcaneous)	Microphthalmia Microcephaly Cleft lip Cleft palate Cardiac (VSD, PDA) Omphalocele Rocker-bottom (protruding calcaneous)
Survival	> 90% die in infancy	> 90% die in infancy



Trisomy 18
(Ovelapping fingers)

Trisomy 13
(Microcephaly, cleft lip & palate)

Autosomal deletion syndromes

4p-
- Prominent forehead
- Broad nasal root [Greek helmet face]
- Short filtrum
- CHD

5p- **(Cri-du-chat syndrome)**
- Microcephaly
- Round face
- Hypertelorism
- Low set ears
- Hypotonia
- Cat-like cry

Sex Chromosome Abnormalities

A) **Turner syndrome (XO)**
B) **Klinefelter syndrome (XXY)**
C) **XXX female**
D) **Fragile X syndrome**

Fragile X Syndrome

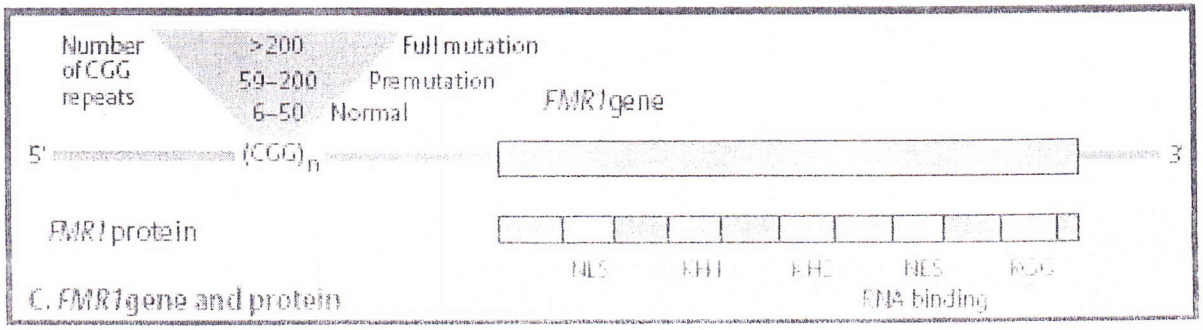
Definition: It is the second most common genetic cause of MR after Down syndrome
Incidence: 1/4000
Mode of inheritance: XL-R (FMR1 gene)
Genetic Defect: Fragile site in the distal part of the long arm of the X chromosome
Molecular analysis: Tri-nucleotide repeat expansion [CGG] that may ↑↑ in size "expansion" through females in successive generations
Clinical Picture:

a. ♂: Mental retardation
Macro-orchidism (When?)
Facies: Long face, large everted ears, broad forehead, prominent mandible
Behaioral: Autistic & / or hyperactive behavior
Cardiac: Mitral valve prolapsed

b. ♀: Variable degrees of MR (usually mild to moderate) & learning disability

Diagnosis: Detection of CGG repeat number (PCR)

	Repeat	Normal	Premutation	Affected
Fragile X syndrome	CGG	< 50	55-199	> 200
Myotonic dystrophy	CTG	5-37	37-50	> 50
Huntington Chorea	CAG	5-37	-	> 40-55



Turner Syndrome

Genetic Types

- ☒ Non-disjunction (most common): The X chromosome is usually of maternal origin
- ☒ Mosaicism (better prognosis): 45, X / 46, XX

Clinical picture

(A) At birth: Edema of dorsum of hands & feet

(B) Childhood:

- Short stature (Mean = 143 cm)
- Webbing of the neck
- Widely spaced nipples
- Cubitus valgus (↑↑ carrying angle)
- Low posterior hair line
- Normal mentality (MR in 18%)
- Cardiac: Coarctation, bicuspid aortic valve
- Renal: Hoarseshoe, ectopic kidney…
- Thyroiditis (30%): Hypothyroidism
- IGT, Type II DM

(C) Puberty:

- Secondary sex characters fail to develop

Investigations

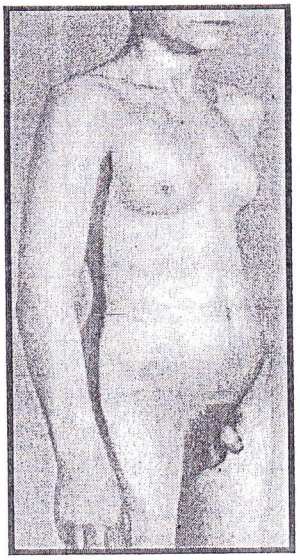
1. Estrogen level: ↓↓
2. Gonadotropins "FSH& LH": ↑↑ (specially > 11 yrs)
3. Karyotyping (45, X)
4. U/S, Echocardiography, Thyroid profile

Treatment

- **hGH**
- **Estrogens:** To induce the development of 2ry sex characters. Start at 11-12 yrs (Why?)
- **Estrogen + Progesterone cyclic therapy:**
  - Estrogen D1- D23
  - Progestrone D10- D23
  - No Rx D23-D30 ⟶ Withdrawal bleeding
- **Ovum donation + IVF: ??** Fertility

	Turner	Noonan
Sex	Only ♀	♀& ♂
Genetics	Non-disjunction	AD
Cardiac	CoA / bicuspid aotic valve	PS
Mentality	MR in 18%	MR more common
Sexual Development	Hypogonadism	Delayed (2yrs)

Klinefelter Syndrome



Incidence 1:600-1.000 liveborn ♂

Genotype 47, XXY
Variants: XXXY, XXXXY (↑↑ Severity)

Etiology Non-disjunction

Clinical picture

- Disproportionate tall stature:
  - Span > height,
  - Lower segment > Upper segment
- Small testes (Prader Orchidometer)
- Gynecomastia (30%)
- Infertility (Azoospermia)

Complications Breast cancer, mediastinal germ cell tumors

Treatment Testosterone replacement therapy

Poly-Y male (47, XYY male)

- No Hypogonadism
- Aggressive antisocial behavior& violence

Triple-X female (47, XXX female)

- No Hypogonadism
- learning& behavioral disorders
- Normal fertility but early menopause is common

Noonan Syndrome:

- Etiology: AD^{12} (variable expression)
- Features of Turner $ + **Normal karyotyping** + affecting ♀& ♂
- Short stature, neck webbing, cubitus valgus
- Cardiac: Cardiomyopathy, PS, branch pulmonary artery stenosis
- Facies: Hypertelorism, epicanthus, ptosis, micrognathia, antimongloid slant, ear anomalies
- Hypogonadism: Delayed puberty (2 yrs later)
  - ♂: Small testes, cryptorchidism
  - ♀: Premature ovarian failure
- Mental retardation: 25-30%
- Rx: hGH

X-Chromosome Inactivation (Lyon Hypothesis)

Inactivation occurs through methylation of cytosine

Sex chromatin (Barr body)

- It appears in individual with ≥ 2 X chromosomes
- No. of sex chromatin = No. of X – 1
- Size of sex chromatin changes with change of the size of X chromosome

Lyon Hypothesis *(after Mary Lyon = 1961)*

1. Sex chromatin in ♀ is genetically **inactive** X chromosome
2. Inactivation starts in the **intra-uterine** life
3. Inactivation is **random** but **fixed** (either paternal or maternal X chromosome and all cells derived from that cell)
4. Females are **mosaic** for X-linked genes

Benefits of Lyon Hypothesis

1. It explains why the gene product of the 2 Xs in ♀ is equal to gene product of 1 X in ♂
2. It explains why in XLR diseases, homozygous ♀ is affected equal to hemizygous ♂
3. It explains why heterozygous ♀ "Carrier" for XLR gene may show clinical / biochemical findings:
   - ☒ Duchenne muscular dystrophy: ↑↑ CK
   - ☒ Hemophilia: ↑↑ PTT
   - ☒ Ocular albinism: Patchy pigmentation of the fundus
   - ☒ Retinitis pigmentosa: Abnormal retinal reflex
4. In XLR diseases, if male is affected; there are one of 2 possibilities:
   - ☒ Mother is carrier: So test the mother for clinical / biochemical findings (as above)
   - ☒ New mutation (Mother is free)

Disadvantages of Lyon Hypothesis

It fails to explain why Turner ♀ with one X is *not* normal, so other theories emerge:

1. Both X chromosomes are active early in IU life to allow normal development of XX
2. Some genes essential for normal development escape inactivation
3. Inactivation starts early but it is gradual allowing normal development
4. Both XX are active but they function as one X chromosome of a male

Gene structure& Function

No. of human genes ≈ 25.000

Gene

- It is a DNA sequence that directs the synthesis of a specific polypeptide chain
- Most of the DNA (> 95%) **does not code** proteins with no known function
- Non-coding sequence lie within (introns) & between genes
- 45% of DNA is formed of repeat sequences (unknown function) called satellites, which vary among individuals "**DNA finger printing**"
- People are genetically very similar (99.9% identical)
- The 0.1% difference is not negligible as it corresponds to 3 millions nucleotides

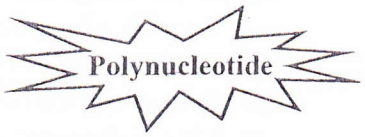
No. of nucleotides ≈ 3 billions

Pseudogene

It is DNA sequence resembling normal gene but has minor changes preventing its transcription

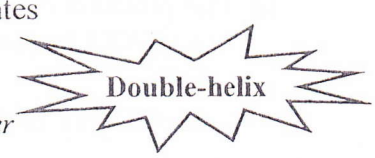
DNA

- It is a polynucleotide, double helix molecule formed of 2 complementary strands
- Nucleotide = Sugar + Phosphate + Nitrogenous base
- Sugar = Deoxyribose
- Nitrogenous bases
  - **a. Pyrimidines:** Thymine (T) & Cytosine (C)
  - **b. Purines:** Adenine (A) & Guanine (G)



- The outer backbone of the helix is formed of alternating sugars & phosphates
- The bases project inwards from the outer sugar-phosphate framework
- The bases are complementary:
  - A = T (2 hydrogen bonds)
  - G = C (3 hydrogen bonds)

  The bonds help to hold the 2 strands together & in the Repair of damaged DNA



- The 2 strands run in opposite directions [one in 5'-3' & the other in 3'-5' direction]
- DNA is present in the nucleus (1% of total cellular DNA is present in the mitochondria)

Functions of DNA

A) Replication

- ☒ It occurs in the interphase before cell division
- ☒ Each strand acts as a template ⟶ synthesis of a new strand
- ☒ The new DNA molecule is formed of 2 strands; one *old* & one *new* [Semiconservative]
- ☒ Replication is catalyzed by ***DNA polymerase***

B) Transcription

- ☒ It is synthesis of mRNA molecule from a DNA template
- ☒ Only one strand acts as template [Template = Antisense]
- ☒ The other strand is called [Sense = Coding strand]??
- ☒ This is not fixed; a given strand may act as template for some genes & coding for others

The coding (Sense) strand has the same sequence of the synthesized RNA except..

C) Translation

- ☒ It is protein synthesis via "translation" of genetic information carried on mRNA
- ☒ It occurs in the ribosomes (Cytoplasm)

Mitochondrial DNA

- The mitochondrion contains 2-10 copies of double stranded DNA (mt-DNA)
- Size = 16 Kb (Kilobase)
- Circular
- No introns
- Exclusively transmitted by the mother [Sperm does not contain mitochondria]
- Slightly different genetic code (e.g., UGA codes for tryptophan not a stop codon)
- Mt-DNA codes for ⟶ 13 proteins (components of the respiratory chain)
  ⟶ 2 rRNA & 22 tRNA
- Mt-DNA mutations lead to mitochondrial diseases e.g., MELAS, MERRF, KSS

RNA

	DNA	RNA
Name	Deoxyribonucleic acid	Ribonucleic acid
Site	Mainly in the nucleus (& mitochondria)	Mainly in the cytoplasm (& nucleus)
Sugar	Deoxyribose	Ribose
Bases	A, G, C, T	A, G, C, U
Strands	Two	One
Type	One	4 types (hnRNA, mRNA, tRNA, rRNA)
Shape	Double helix	Variable

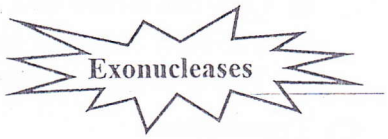
Organization of genes

- ☒ Exon: a segment of the gene that is represented in the final spliced mRNA
- ☒ Intron: a segment of the gene that is not represented in the final spliced mRNA because it has been removed during splicing of exons around it
- ☒ Boundaries between exons & introns are not random. Introns begin with GT & end with AG
- ☒ The gene is formed of Promoter, Transcription unit & Terminator
- ☒ **RNA polymerase (RNAP)** binds to the promoter
- ☒ The promoter contains:
  - **a. TATA boxes:** 25 nucleotides upstream (at the 5') the transcription unit
    It allows **attachment** & **separation** of RNAP to the promoter
  - **b. CCAAT boxes:** 70 nucleotides upstream (at the 5') the transcription ~~unit~~
    It determines the **frequency** of transcription
- ☒ The promoter also contains:
  - **a. Enhancers:** ↑↑ Gene expression
  - **b. Silencers:** ↓↓ Gene expression

Transcription

- ☒ It is synthesis of mRNA molecule from a DNA template (RNAP enzyme)
- ☒ Only one strand acts as template, the other strand is called the coding strand
- ☒ RNAP attaches to the promoter
- ☒ TATA box: Initiation of transcription
- ☒ CCAAT box: Frequency of transcription
- ☒ The primary transcript RNA undergoes the following modifications (Processing):

(4)
1. Capping of 5' by GTP
2. Methylation
3. Addition of Poly-A tail
4. Removal of introns & splicing of exons



Translation

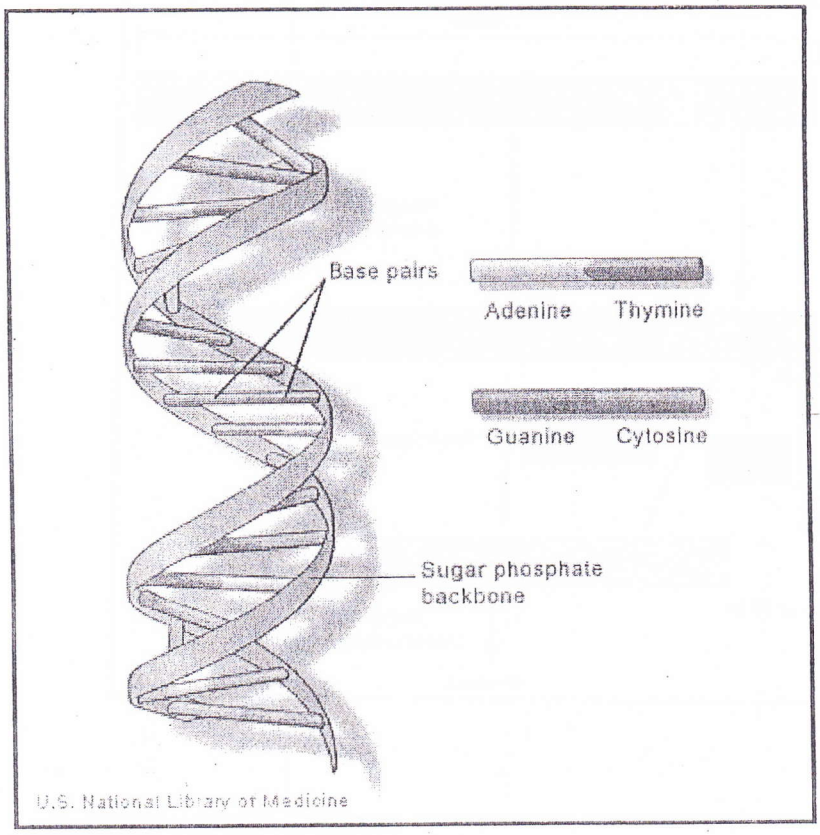
- ☒ It is protein synthesis via "translation" of genetic information carried on mRNA
- ☒ It occurs in the ribosomes (cytoplasm)
- ☒ The ribosome moves along mRNA in the 5'-3' direction, translating the successive codons
- ☒ The 1st codon to be translated is AUG (coding for methionine)
- ☒ tRNA brings the amino acids to mRNA-ribosome complex
- ☒ The anticodon on tRNA can recognize the complementary codon in the mRNA
- ☒ The process is repeated in 5'-3' direction till reaching a stop codon
- ☒ The polypeptide is released

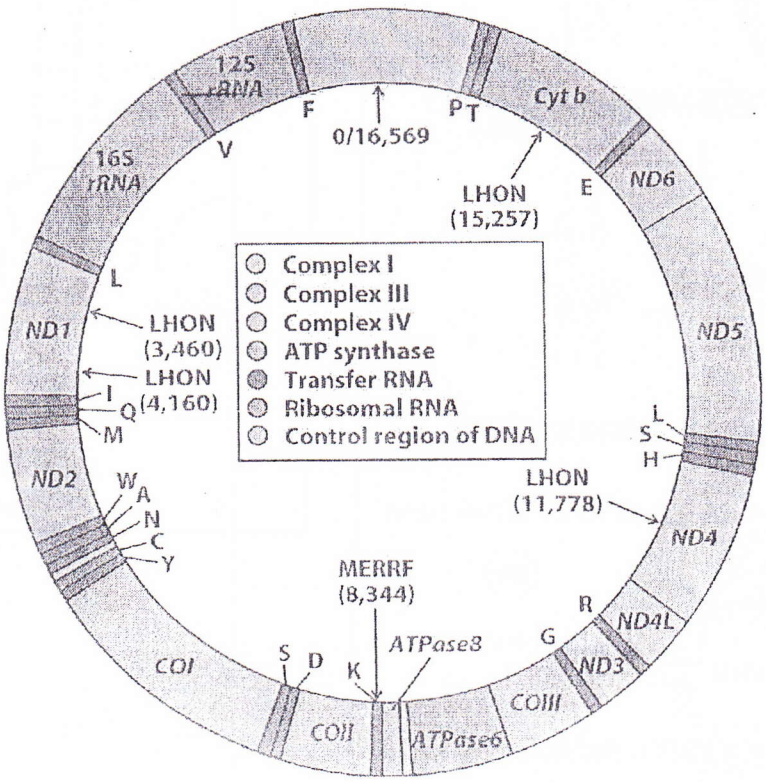
Protein structure

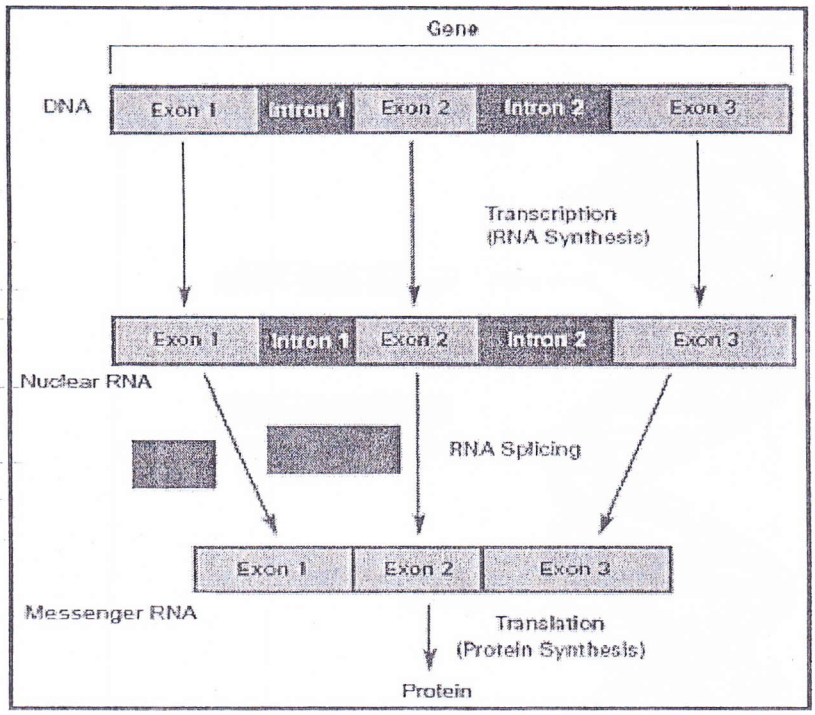
- Primary: Polypeptide chains
- Secondary: Change in the configuration of the protein due to bonding between groups (e.g., hydrogen bonds lead to twisting into helix)
- Tertiary: 3-dimentional shape

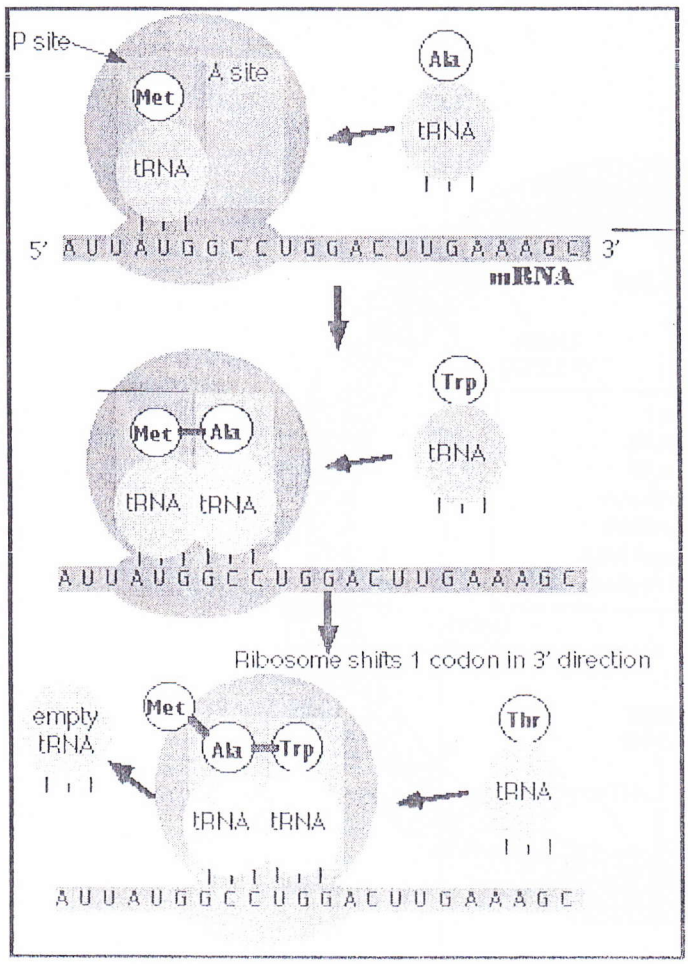
Control of gene expression *(All cells contain All genes necessary for every cell function)*

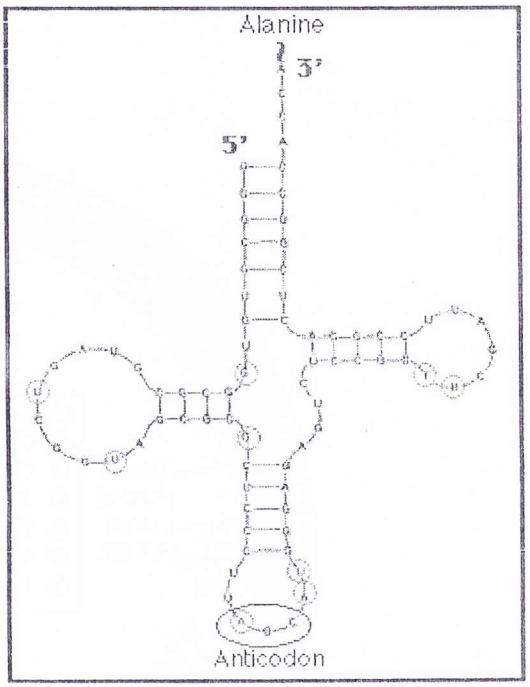
- ☒ TATA & CCAAT boxes
- ☒ Enhancers & Silencers [Either ***cis:*** near by the gene or ***trans:*** away from on other chromosome]
- ☒ Intronic sequence
- ☒ Methylation of genes (Methylation of the gene ⟶ ↓↓ Gene expression)
- ☒ Sequence 3' to the gene


Base pairs
Adenine
Thymine
Guanine
Cytosine
Sugar phosphate backbone
U.S. National Library of Medicine


12S rRNA
16S rRNA
F
V
0/16,569
PT
Cyt b
LHON (15,257)
E
ND6
L
ND1
LHON (3,460)
LHON (4,160)
I
Q
M
Complex I
Complex III
Complex IV
ATP synthase
Transfer RNA
Ribosomal RNA
Control region of DNA
ND5
L
S
H
ND2
W
A
N
C
Y
LHON (11,778)
ND4
MERRF (8,344)
R
ND4L
G
ND3
S
D
K
ATPase8
COI
COII
ATPase6
COIII


Gene
DNA
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Transcription
(RNA Synthesis)
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Nuclear RNA
RNA Splicing
Exon 1
Exon 2
Exon 3
Messenger RNA
Translation
(Protein Synthesis)
Protein


P site
A site
Met
tRNA
Ala
tRNA
5' A U U A U G G C C U G G A C U U G A A A G C 3'
mRNA
Trp
Met
Ala
tRNA
tRNA tRNA
tRNA
A U U A U G G C C U G G A C U U G A A A G C
Ribosome shifts 1 codon in 3' direction
empty
tRNA
Met
Ala
Trp
tRNA tRNA
Thr
tRNA
A U U A U G G C C U G G A C U U G A A A G C


Alanine
3'
5'
Anticodon

Genetic code (Codon)

Definition

It is a sequence of 3 adjacent nucleotides in a nucleic acid that code for one amino acid

Characteristics of the Codon

1. **Triplet** (3 adjacent nucleotides). We have 64 codons & 20 amino acids
2. Written in the **direction** 5'-3'
3. **Specific**: Certain codon always code for only one specific amino acid
4. **Universal**: applied to all organisms (Mitochondrial DNA has a slight different codons)
5. **Redundancy** (Degeneracy): a given amino acid may have more than one codon
6. **Initiating** codon = AUG (which codes for methionine)
7. **Stop** codons = UAG, UGA, UAA
8. The 3rd base of the codon is the least important e.g., Glycine has GGG, GGC, GGU, GGA
9. Amino acids with similar chemical properties have related codons (Codons with U in the middle are hydrophobic)
10. The mRNA codon is recognized by **complementary anti-codon** (a sequence of 3 adjacent nucleotides in the middle loop of tRNA molecule)

Mutations

Definition

Changes in the nucleotide sequence as a result of mutagen exposure (may be spontaneous)

Classification

I) **Point mutation (base substitution):**
- ☒ Transition: Pyrimidine ⟷ Pyrimidine or Purine ⟷ Purine
- ☒ Transversion: Pyrimidine ⟷ Purine

Effects of point mutations:

1. **Silent** mutation: new codon but for the same amino acid (e.g., GGC → GGA)
2. **Nonsense** mutation: if the resulting codon is a stop codon (e.g., AGA → UGA)
3. **Sense** mutation: if a stop codon is changed into another coding one (e.g., UGA → AGA)
   Hb constant spring (α chain with 31 extra amino acids)
4. **Missense** mutation: different amino acid is formed. This change may be:
   a. Acceptable: No change in protein function
   b. Partially acceptable: Valine replaces glutamic acid in the β-chain of Hb resulting in the formation of HbS which still can carry O_2
   c. Unacceptable: HbM (Can not carry O_2)
   d. Splicing mutation: Failure of splicing of exons

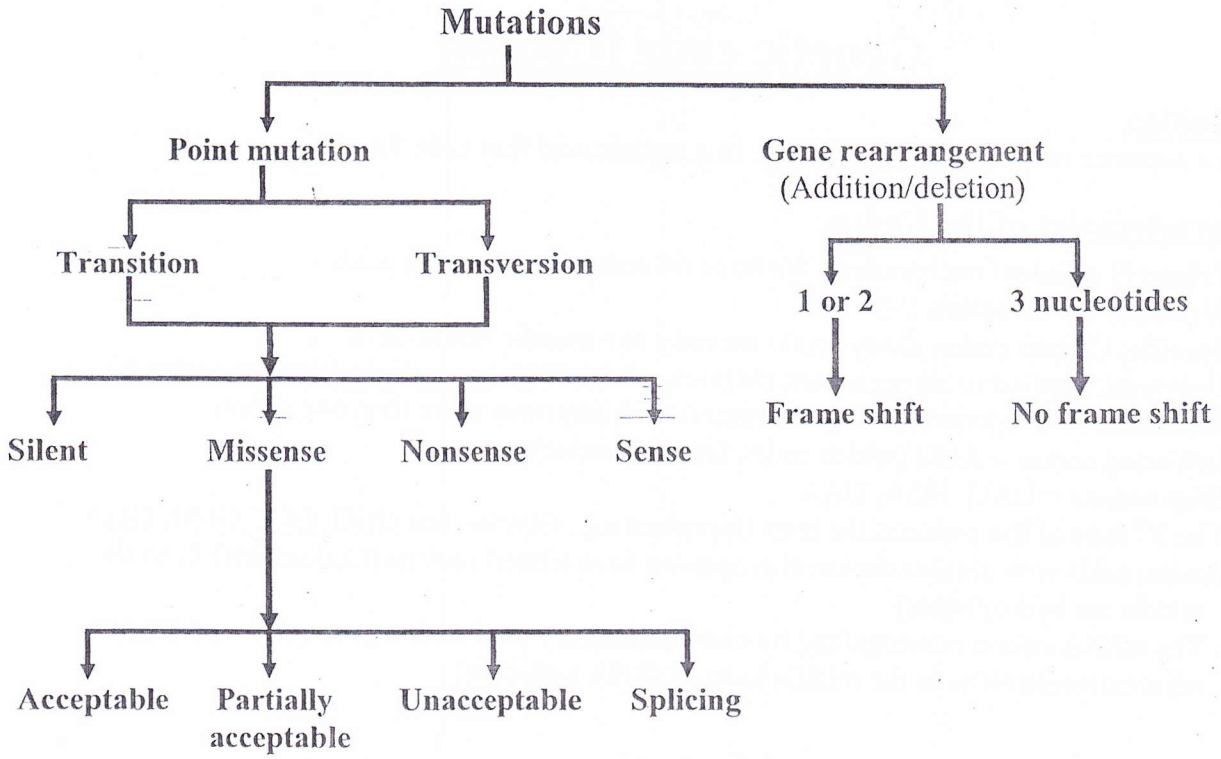
II) **Addition or Deletion of nucleotides (Gene rearrangement):**
- ☒ One or two nucleotides: Frame shift (change of all codons on the 3' side)
- ☒ Three nucleotides: No frame shift (Addition or Deletion of one amino acid)

Etiology *(Mutagens)*

- ☒ Ionizing & non-ionizing radiation
- ☒ Chemicals e.g., alkylating agents
- ☒ Inherited diseases?? ⟹
- ☒ Spontaneous

Types or mutation

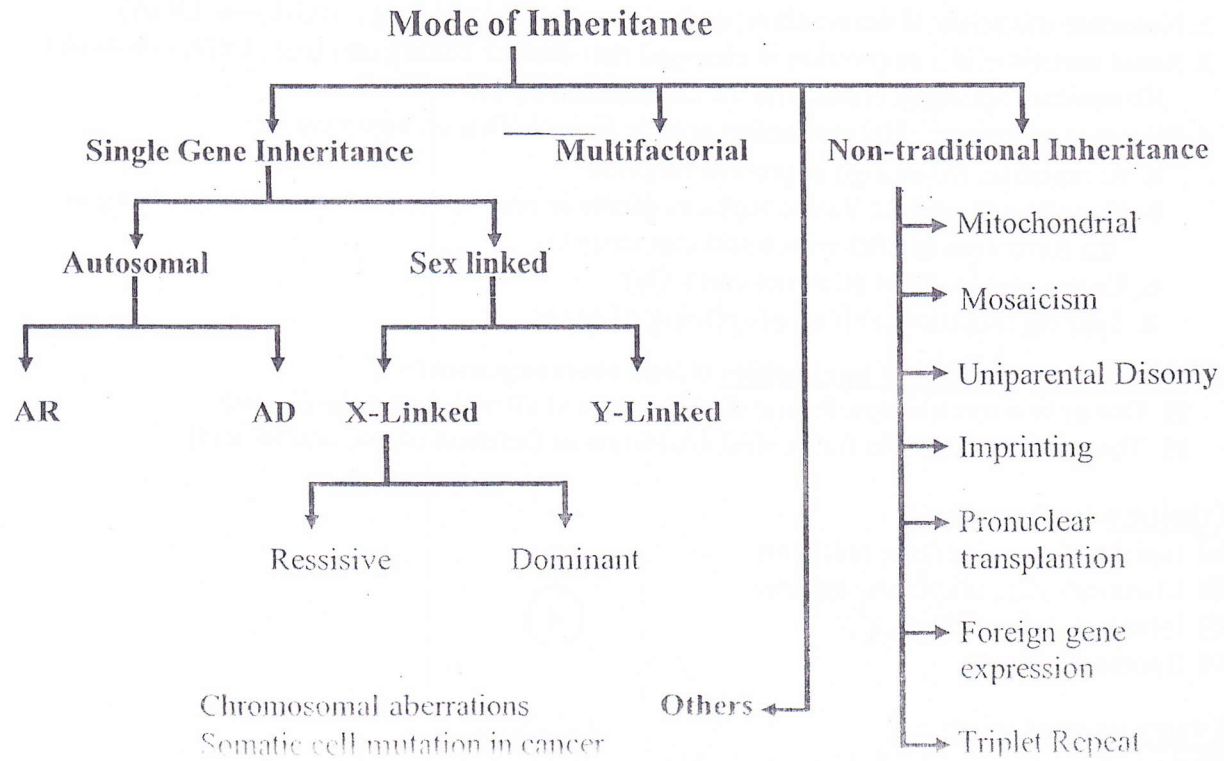
- ☒ Mutation of somatic cells: Non-heritable
- ☒ Mutation of germ cells: Heritable



Classes of DNA

	Repeat size (bp)	Total size (kb)	Features
Satellite	5-200	100	
Minisatellite	10-60	20	2 families; VNTR & telomeric family
Microsatellite	1-4	1	Repeats of A, CA

(VNTR = Variable number of tandem repeats)



Mendel's Laws:
1. Unit Inheritance
2. Segregation
3. Dominance
4. Independent assortment

Mode of Inheritance

A) Single Gene Inheritance

Gene
It is a DNA sequence that directs the synthesis of a specific polypeptide chain

Locus
It is the site of a gene on a chromosome

Allele
It is alternative form of a gene found at the same locus on a chromosome. Alleles on homologous chromosomes may be:

a. Homozygous: identical (they may be normal or not)
b. Heterozygous: different
c. Hemizygous: there is only one allele for genes on X or Y chromosomes in males

Dominant allele
It expresses itself whether homozygous or heterozygous

Recessive allele
It expresses itself only if homozygous

Codominance = Both alleles are expressed in the heterozygous

Autosomal Inheritance

	Autosomal Dominant	Autosomal Recessive
Affected Individual	▪ May be homozygous or heterozygous ▪ One parent must be affected (except for new mutation)	▪ Must be homozygous ▪ Both parents are heterozygous (carrier) ▪ Parental consanguinity → ↑↑ incidence
Sex	♀ & ♂ are equally affected	♀ & ♂ are equally affected
Carrier	No carries state	Carrier (phenotypically normal)
Transmission	Vertical (No skipping)	Transverse (skipping)
Offsprings	▪ Homozygous parent → 100% affected ▪ Heterozygous parent → 50% affected ▪ Heterozygous parents → 75% affected	▪ Homozygous parent → 100% carrier ▪ Heterozygous parents → 25% affected, 25% normal, 50% carrier
Defect	Structural	Functional
Severity	Usually less severe	Usually more severe
Examples	Huntington chorea, Myotonic dystrophy Neurofibromatosis, Tuber sclerosis Facioscapulohumeral muscular dystrophy Osteogenesis imperfecta, Achondroplasia Marfan, Ehler-Danlos, ADPKD, H.spherocytosis	Gaucher, Galactosemia, phenylketonuria, Homocystinuria, Hurler, albinism, SMA, CAH, Cystic fibrosis, Wilson, Cystinosis, GM_1, GM_2 (Tay-Sachs, Sandhoff), Zellweger
Pedigree	**Aa X aa** **Aa Aa** (50% Affected) **aa aa** (50% Normal)	**Aa X Aa** **AA** 25% Normal **Aa Aa** (50% Carrier) **aa** 25% Affected

Sex Linked Inheritance

A) Y-Linked Genes (Holandric Inheritance):

- The trait is transmitted only in ♂ (♂ to ♂ transmission)
- Affected male has all his sons affected
- Examples: Hairy pinna

B) X-Linked Genes:

1. X-linked Dominant:

- The same as AD transmission but:
- Affected ♂ transmits the trait to all of his daughters but **none** of his sons
- May be lethal in ♂

2. X-linked Recessive:

- Affected ♂ is hemizygous
- ♀ are affected (Homozygous) in the following conditions: ⟹

Affected ♀ in XLR:
1. ♀carrier + affected ♂
2. Turner
3. Lyonization

	X-linked Dominant	X-linked Recessive
Affected Individual	▪ May be homo-, hetero- or hemi- ▪ One parent must be affected (except for new mutation)	▪ Homozygous ♀ or hemizygous ♂ ▪ Affected ♂ Mother is obligate carrier ▪ Affected ♀: When?
Sex	Heterozygous ♀ is affected	Heterozygous ♀ is carrier (♂>♀)
Carrier	No carries state	Carrier (phenotypically normal ♀) No carries in ♂ (hemizygous)
Transmission	Vertical (No skipping)	Transverse (skipping)
Offsprings	▪ Homozygous ♀ ⟶ 100% affected ▪ Heterozygous ♀ ⟶ 50% affected ▪ Hemizygous ♂ ⟶ 100% of ♀ affected 0 % of ♂ affected (**none**…)	▪ Heterozygous ♀ (carrier) ⟶ 50% of sons affected 50% of daughters carrier ▪ Affected ♂ Hemizygous ⟶ 100% of daughters carrier 0% of sons affected (**none**…) i.e., all are phenotypically normal
Examples	Hypophosphatemic rickets Alport syndrome Incontinentia pigmenti	Hemophilia A & B, G-6-PD deficiency, Duchenne, Hunter, Color blindness, Menkes, Fabry, Nephrogenic DI, CGD Lesch-Nyhan, Wiskott-Aldrich, Fragile X
Pedigree	XX x XY XX XY (50% Affected) XX XY (50% Normal)	XX x XY XX XX (25% Carrier) XY XY (25% Affected)

B) Multifactorial (Polygenic)

Definition: It is interaction between **polygenic& environmental** factors

Characteristics:

1. **Familial**
2. Has **sex** preference (CHPS is more common in ♂)
3. RR is the **same** for relatives who share equal proportion of genes (1st degree relatives)
4. RR is ↑↑ in **consanguineous** parents
5. RR is ↑↑ if the relationship to affected relative is **close**
6. RR is ↑↑ if the disease in the affected relative is more **severe**
7. RR is ↑↑ if **more** than one close relative is affected
8. RR is ↑↑ if there is affection of the less frequently **affected sex** (♀ relative with CHPS)
9. The concordance rate in **monozygotic** twins is not 100% (20-60%)
10. The concordance rate in **dizygotic** twins is 2-4% (similar to ordinary siblings)

Examples: CHD, CHPS, DDH, talipes, spina bifida, DM, obesity, HTN, epilepsy, asthma

C) Non-Traditional Inheritance

1. Mitochondrial (Cytoplasmic)

- ☒ Mitochondrial DNA (discuss)
- ☒ The ♂ child of affected mother does not transmit the trait

2. Mosaicism

Post-fertilization event

Definition: It is the presence of ≥ 2 different cell lines derived from 1 fertilized ovum

Types:

a. Somatic cell mosaicism:

- Gene mutation in somatic cells
- The mutation is transmitted to daughters cells not to offsprings **"Non-heritable"**
- Serious if it occurs early in embryogenesis

b. Germ cell mosaicism:

- Gene mutation in germ cells
- The mutation is transmitted to offsprings **"heritable "**
- Diagnosis is difficult (Suspected when more than one affected child is born)

3. Uniparental disomy (UPD)

Definition: It is the inheritance of 2 homologous chromosomes (or 2 copies of gene) from one parent

Types:

a. Isodisomy: Duplication of a chromosome in case of monosomy

b. Heterodisomy: Loss of a chromosome in case of trisomy

Significance: a child may be affected with AR trait if only one parent is carrier (isodisomy)

Examples:

- ☒ Prader-Willi syndrome occurs due to:
  Deletion* of paternally acquired segment of chromosome 15
  Or maternal disomy (both chromosomes 15 are from the mother)
- ☒ Angelman syndrome occurs due to:
  Deletion* of maternally acquired segment of chromosome 15
  Or paternal disomy (both chromosomes 15 are from the father)
- ☒ Cystic fibrosis: 1/500 of cases are due to UPD (del F508)
- ☒ Beckwith Wiedmann syndrome

Normally the 2 copies of most genes are functionally equivalent

4. Genomic imprinting

Definition: It is different gene expression depending on the parent of origin of such genes

Mechanism: Inactivation (imprinting) of genes occurs through methylation

Examples:

- ☒ Prader-Willi &Angelman $: chromosome 15 is dependent on parental origin
- ☒ Huntington chorea: if the gene is transmitted from the father ⟶ severe juvenile form
- ☒ Myotonic dystrophy: „ „ „ „ „ the mother ⟶ severe congenital form

5. Pronuclear transplantation

- ☒ Experimentally in mice

  Zygot formed of 2 sets of paternal chromosomes ⟶ well developed placenta & membranes
  Poor development of embryonic structures

  Zygot formed of 2 sets of maternal chromosomes ⟶ well developed embryonic structures
  Poor development of placenta& membranes

- ☒ In humans

  2 sets of paternal chromosomes ⟶ Hydatidiform mole (placental tumor)
  2 sets of maternal chromosomes ⟶ Teratoma (embryonic tumor)
  2 sets paternal + 1 maternal ⟶ Large placenta + IUGR
  2 sets maternal + 1 paternal ⟶ Small placenta + IUGR (placental insufficiency)

6. Foreign gene expression

If a foreign gene is inserted very early in the embryo, it will be transmitted to offsprings
But expressed only if it was transmitted from the father

7. Triplet Repeat Expansion disorders

Definition: Diseases caused by expansion of the number of 3-base-pair repeats (e.g., CGG)
Mechanism: expansion of the number is a dynamic process through successive generations
Examples:

- ☒ Fragile X syndrome (XL-R)
- ☒ Huntington chorea (AD)
- ☒ Myotonic dystrophy (AD)

Anticipation

Variation in Gene Expression

1. Penetrance

The proportion of individuals with a particular genotype to have the corresponding phenotype
Examples: Achondroplasia, Hereditary spherocytosis

2. Expressivity

Variation in the severity of expression of a particular gene
Examples: Osteogenesis imperfecta, Hereditary spherocytosis

3. Anticipation

The tendency of some diseases to have an earlier onset & / or increased severity with successive generations
Examples: Huntington chorea, Myotonic dystrophy

4. Pleiotropy

Multi-system affection in spite of single gene affection
Examples: Galactosemia, Tuberous sclerosis

5. Heterogeneity

The same C/P is caused by affection of more than one gene (**each one** can cause the disease)
Examples: Homocystinuria

6. Multifactorial Inheritance

The interaction between polygenic & environmental factors
Examples: CHD, CHPS, DDH, talipes, spina bifida, DM, obesity, HTN, epilepsy, asthma

7. Gene Interaction

The expression of one gene is affected by the presence of other genes
Examples: Bombay phenotype of blood groups

8. Linkage

The co-segregation of 2 non-allelic genes which have their loci very close to each other on the same chromosome & so they move together during meiosis
Examples: Genetic markers used in detection of a particular gene

9. Sex influences

The effect of the gender on the C/P of some diseases
Examples: XL diseases

10. Variation of Age of Onset

The variation in the onset of presentation of some diseases
Examples: Myotonic dystrophy (Early), Huntington chorea (Late), Hemochromatosis (Late)

Control of gene expression

- ☒ TATA & CCAAT boxes
- ☒ Enhancers & Silencers
- ☒ Intronic sequence
- ☒ Methylation of genes
- ☒ Sequence 3' to the gene

Genetic Engineering
Recombinant DNA Technology

Definition

It is the science that deals with the detailed **structure** of genes together with their normal physiological **function**, pathological **defects** & possible **treatment**. It usually involves the formation of recombinant DNA molecule

Recombinant DNA

It is the creation of a new DNA by cutting of DNA segment from parent genome & joining it to another DNA molecule (Vector)

Requirements: 2 Enzymes; restriction endonuclease, DNA ligase, Vector, Bacteria for cloning

Vector

It is a DNA molecule used to carry DNA region of interest

Type	Nature	Can carry up to
Plasmid	▪ Small extra-chromosomal circular double stranded DNA molecules ▪ Found in bacterial cells, but multiply independent on the cell ▪ Carry some genes (e.g., antibiotic resistance…)	10 Kb
Phage	Virus that infects bacteria	20 Kb
Cosmid	Plasmid containing part of phage	50 Kb

Restriction Endonucleases

Bacterial DNA is methylated

- ☒ These are **bacterial** enzymes
- ☒ Restriction: they **restrict** the multiplication of bacteriophage viruses in the bacterial cells
- ☒ Endonucleases: they **cut** in the **middle** of the polynucleotide chain at specific sequences
- ☒ Restriction (recognition) sites: **specific** sequence at which cutting occurs. Restriction sites are usually **symmetrical** e.g., 5' GAATTC 3' ['Madam = I'm Adam]
- ☒ Products of restriction enzymes: Fragments with different length with sticky or blunt ends
- ☒ Number: > 2000 enzymes have been isolated
- ☒ Examples: EcoRI, Mst II…

Ligase

It is an enzyme which can join 2 DNA molecules usually derived from bacteria (E.coli)

Technique

I) Preparation of gene:

1. Intact genes: using restriction enzymes → large fragments with large number of genes
2. Synthesis of DNA from mRNA (complementary DNA = cDNA)

   mRNA → (Reverse transcriptase) → cDNA (single strand) → Double stranded DNA

   This DNA contains only the coding sequence (No introns- No regulatory elements)
3. Chemical synthesis: for small genes

II) Formation of Recombinant DNA

1. Separation of the **plasmid** from host cell (bacteria)
2. **Opening** of the circular DNA of the plasmid by a restriction enzyme
3. **Human DNA** is also broken by the same restriction enzyme& the part containing the required gene is separated
4. **Insertion** of the separated gene into the plasmid
5. The **circle** is reformed by the enzyme DNA ligase (chimeric molecule is formed)
6. The plasmid is reinserted into the **bacterial cell**
7. **Culture** on a medium containing antibiotic to which plasmid is resistant

8. Selective growth & **multiplication** of bacteria containing the recombinant plasmid
9. The bacteria can also produce human **protein** coded by the human gene
10. **Extraction** of plasmid from bacterial cell
11. **Opening** of the plasmid by a restriction enzyme
12. Separation of multiple copies of human DNA **(synthesis of DNA probes)**

DNA Probes

Definition

It is a labeled single stranded DNA fragment which can hybridize specifically with complementary sequences helping in their identification. Labeling is done by fluorescence or radioactive ^{32}P

Types

- ☒ Gene specific probes
- ☒ Complementary DNA (cDNA) probes
- ☒ Synthetic probes

Synthesis

Recombinant DNA technology

Applications of Recombinant DNA Technology

1. Gene structure, function & mapping
2. Diagnosis of genetic diseases (& understanding of pathogenesis) ⟹
3. Clinical genetics
   - ☒ Prenatal diagnosis
   - ☒ Presymptomatic diagnosis
   - ☒ Carrier detection
4. Synthesis of genetically engineered vaccines
5. Biosynthesis of: Insulin, GH, EPO, Factor VIII, GM-CSF, Interferon
6. Gene therapy
7. Agriculture??

Examples of diseases:
1. β-Thalssemia
2. Sickle cell
3. Cystic fibrosis
4. α_1- antitrypsin deficiency
5. Gaucher
6. Tay-Sachs

Genetically engineered vaccines

Advantages Low cost No contamination ↑↑ Antigenicity ↓↓ potential infectivity

Strategies

Strategies for live attenuated vaccines	Strategies for live inactivated vaccines
A) DNA modification ▪ Attenuation of the organism, followed by: ▪ Genetic modification preventing reversion to the wild virulent form **B) Recombinant viruses** **a.** Recombinant Influenza vaccine **b.** Recombinant vaccinia virus vaccine ▪ Removal of the non essential DNA sequences of vaccinia virus and ▪ Replacing them by certain genes responsible for antigenicity of other pathogens ▪ Single vehicle ⟶ vaccines against many pathogens	**A) Synthetic peptide** Use of short segment of the protein (rather than the entire molecule) as the immunogen **B) Recombinant HBV vaccine** ▪ Isolation of the viral gene coding for HBsAg ▪ Insertion into baker's yeast ▪ Synthesis of ↑↑ amount of HBsAg ▪ Purification ▪ Inactivation with formalin ▪ Adsorption on $Al(OH)_3$ ▪ Storage at 2-8° C

Diagnosis of Genetic Diseases & DNA Polymorphism

Isolation

1. Type of the cell: WBC, lymphocytes, skin fibroblasts, amniocytes, chorionic villi cells
2. Detergent + cell [Dissolves lipids]
3. Sodium dodeyl sulphate (SDS) [Librates proteins]
4. Shaking with Phenol [Coagulates proteins]
5. Ethyl alcohol [Precipitates DNA]

Techniques *(All are done using specific probes)*

1. Blotting & Hybridization

It allows visualization of a specific fragment

Types:

- ☒ Southern blotting for DNA
- ☒ Northern blotting for RNA
- ☒ Western blotting for Proteins

Disadvantages:
1. Long time
2. Large amount is needed
3. Radioactivity

In Southern blotting, the DNA is first digested by specific restriction enzyme into fragments ⟶ Gel electrophoresis into bands ⟶ Addition of specific DNA probes to the bands ⟶ Detection of complementary strands in the sample *(if present)*

2. Polymerase Chain Reaction (PCR)

It allows massive amplification of a specific DNA segment. PCR is done in cycles
One segment can be amplified up to 1 million within 2-3 hours

Requirements:

- Thermostable DNA polymerase
- 2 Oligonucleotide primers (20-30 nucleotide long) that can hybridize to complementary sequences on the DNA strands (one primer for each DNA strand) flanking the area of DNA to be amplified
- Nucleotides

Cycles:

- **Denaturation**: Heating of the sample (95° C) to separate DNA strands
- **Annealing**: The 2 primers are added + cooling (to allow hybridization)
- **Extension**: Extension of the primers along the 2 DNA strands leading to duplication of the segment of interest
- **Cycles are repeated**: 25-35 times

Product: If there is mutation of the target DNA, amplification can still occur (further analysis)

3. Ligase Chain Reaction (LCR)

It allows massive amplification of a specific DNA segment like PCR. It is done in cycles.
It is a process of ligation & not extension

Requirements:

- Thermostable DNA ligase
- 4 Oligonucleotide primers (20-30 nucleotide long) that can hybridize to complementary sequences on the DNA strands (2 primers for each DNA strand)
- Nucleotides

Cycles:

- **Denaturation**: Heating of the sample (95° C) to separate DNA strands
- **Annealing**: The 4 primers are added + cooling (to allow hybridization)
- **Ligation**: Ligation of the 2 primers along the DNA strand
- **Cycles are repeated**: 25-35 times

Product: If there is mutation of the target DNA, amplification can not occur (except with specific primers)

4. DNA Sequencing

Determination of the exact sequence of nucleotides in the DNA molecule

- 4 reaction tubes are used, to each a mixture of "nucleotides & DNA polymerase" is added
- One dideoxynucleotides of either (A,T,G,C) as chain terminator is added to each tube
- Each tube will contain sequences of different lengths but all terminate with a particular ddNTP
- Electrophoresis of these fragments will help in the detection of the exact sequence

5. Allele Specific Oligonucleotide Probe Analysis (ASO)

Two synthetic oligonucleotides are synthesized one for the normal& the other for mutant gene

- Homozygous normal individual ⟶ Hybridization only with normal oligonucleotide
- Homozygous diseased individual⟶Hybridization only with mutant oligonucleotide
- Heterozygous individual ⟶Hybridization with both normal & mutant oligonucleotides

6. Single Stranded Complementary Polymorphism

It allows rapid screening of a specific gene or DNA variation (polymorphism)

7. Restriction Fragment Length Polymorphism

Rationale:

- Restriction endonucleases cut the DNA at specific sequences (restriction sites) ⟶ Large number of restriction fragments of different lengths
- Any change in the sequence within the restriction site will block cutting ⟶change of the fragment length

Example:

- Mst II is a restriction endonuclease that cuts the DNA at β^6 position
- Mutation causing sickle cell changes the sequence at the restriction site [GAG ⟶ GUG]
- Normal individuals ⟶ 1.1 Kb restriction fragments
- Sickle cell anemia ⟶ 1.3 Kb restriction fragments

Glutamic ⟶ Valine

8. Indirect DNA Diagnosis (Linkage)

- The use of a genetic marker (e.g., DNA polymorphism…) to identify a specific gene
- It requires a family with more than one affected member
- Markers that are closely linked to the affected gene are found much more common in the affected members (diseased)
- Markers that are not associated with the disease are randomly shared between affected & unaffected members of the familys

9. Amplification Refractory Mutation System (ARMS)

Rationale:

- PCR amplifies both normal & mutant genes (further analysis is needed)
- ARMS is allele specific PCR (amplification of a specific allele)

Requirements:

Three oligonucleotide primers are synthesized:

1. One for the normal gene
2. One for mutant gene
3. One is constant & attached to the other complementary DNA strand

Two tubes are used:
1. Tube (A): 1 + 3
2. Tube (B): 2 + 3

Results:

- Amplification in tube (A) only ⟶ Homozygous normal individual
- Amplification in tube (B) only ⟶ Homozygous diseased individual
- Amplification in tube (A) & (B) ⟶ Heterozygous individual

	PCR	LCR
Primers	2 primers (one on each DNA strand)	4 primers (2 on each DNA strand)
Process	Extension	Ligation
Amplification	Normal &Mutant genes (except ARMS)	Normal genes (except if specific primers are used)

Cytogenetics

Definition

It is the study of chromosomes: No., size, shape, structure, inheritance & abnormalities

Techniques

1. Chromosomal Banding

Staining of chromosomes: G banding (Giemsa stain) Q banding (quinacrine)

2. FISH (Fluorescence In Situ Hybridization)

It is fluorescence labeled DNA probes which can hybridize specifically with complementary sequences helping in their identification. It is used to study:

- ☒ Complete chromosomes: Trisomy 21
- ☒ Chromosomal subregions
- ☒ Microdeletion syndromes *(Mention)*

3. Multicolored FISH (M-FISH)

Using different fluorochromes

4. Comparative Genomic Hybridization (CGH)

It finds differences in gene copy number by comparing one genome to another

Technique:

- Reference DNA: Labeled with fluorescence (Red)
- Test DNA: Labeled with fluorescence (Green)
- Mixture of both is added to normal DNA (Not labeled with fluorescence)
- Green/Red ration is measured on each chromosome

Results:

- Region of amplification in test DNA ⟶ Excess of green fluorescence [e.g., Tumor cells...]
- Region of deletion in test DNA ⟶ Excess of red fluorescence [e.g., Deletion syndromes...]
- If test & reference DNA are equally represented ⟶ Green: red ratio = 1:1 "Yellow"

5. Different Display Analysis

It demonstrates difference in gene products between normal & abnormal cells

6. DNA Microarray Technology

Computer analysis of genes

Human Genome Project

No. of human genes ≈ 25.000

Definition

It is an organized coordinated effort to *map* & *sequence* all the human DNA
Started in 1990, the first map released in 1994 (linkage map)
The final version 2004 (covers 99% of the human genome with 99.9% accuracy)

Goals of HGP:

1. Physical map of chrom.
2. Complete a genomic map
3. Find all genes
4. Sequence of all genes
5. Acquire the genome as colonies

Goals of HGP

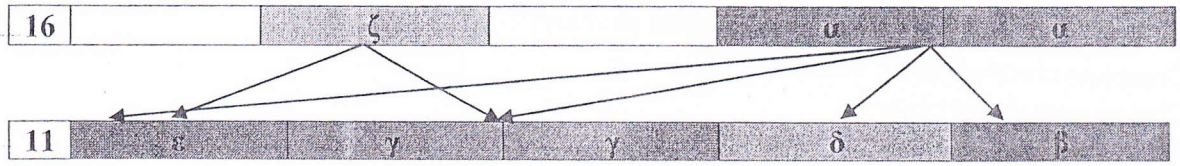
Different Maps

1. **Chromosomal Map:** The number, size & shape of chromosomes in the human cell
2. **Physical Map:** Staining of chromosomes: G banding & Q banding
3. **Linkage Map:** Determine the relative location of genetic markers *(Definition of linkage??)*
4. **cDNA Map:** Shows the position of exons along the DNA
5. **Macrorestriction Map:** Cutting of human DNA with restriction endonucleases into large fragments which are ordered & subdivided into small pieces to be mapped

Genetic Control of Hemoglobin

Physiology

- Hemoglobin is formed of:
  - **a.** Heme (= iron Fe^{++} protoporphyrin): Not genetically determined
  - **b.** Globin: 4 polypeptide chains (2α + 2 non α); each chain contains a heme group
- Globin part is genetically determined. Two families of genes are responsible:
  - **a. α-Gene family** (2 α genes & 1 ζ gene) ⟶ 141 aa
  - **b. β-Gene family** (β gene, δ gene, 2 γ genes & ε gene) ⟶ 146 aa



Normal Hemoglobin

Normal Hb	Name	Structure
Embryonic Hb	Gower I	2 ζ + 2 ε
	Gower II	2 α + 2 ε
	Portland	2 ζ + 2 γ
Fetal Hb	Hb F	2 α + 2 γ
Adult Hb	HbA	2 α + 2 β
	HbA_2	2 α + 2 δ

- Zeta (ζ) & Epsilon (ε) genes stop working by the 3rd month of pregnancy
- By the 3rd month of pregnancy, Hb F is the major Hb

	At Birth	6-12 months
Hb A	20-30 %	97-98 %
Hb F	70-80 %	0-2 %
Hb A_2	-	2- 3.4 %

Abnormal Hemoglobin

Abnormal Hb	Structure
Hb S	2 α + 2 $\beta^{6\ valine}$
Hb C	2 α + 2 $\beta^{6\ lysine}$
Hb D	Variable
Hb E	2 α + 2 $\beta^{26\ lysine}$
Hb H	4 β
Hb Barts	4 γ
Hb M	↑↑ tendency to oxidation (↑↑ Met Hb formation)
Hb Lepore	Fusion of β & δ genes
Hb constant spring	↑↑ α chain by extra 31 aa (sense mutation)

- During fetal life& early childhood, HbF& HbA (β & γ) are inversely proportionate
- 3 months before birth, ↓↓ γ & ↑↑ β synthesis ***(Hb switch)***

Classification of Hb Disorders

A) Qualitative (Hemoglobinopathy) [Structural defects]

a. Point Mutation

☒ Sickle cell anemia Hb S
- Homozygous SS ***(sickle cell anemia)***: [2 α + 2 $\beta^{6\ valine}$] No HbA, HbS 80-95, HbF 2-20%
- Heterozygous AS ***(sickle cell trait)***: [2 α + β + $\beta^{6\ valine}$] HbA 60 % & HbS 40 %

☒ Hb C [2 α + 2 $\beta^{6\ lysine}$]

☒ Hb E [2 α + 2 $\beta^{26\ lysine}$]

☒ Hb M [*Tyrosine instead of histidine*]

b. Deletion: Hb Lyon (Short β chain)

c. Insertion: Hb Grady (Long α chain)

d. Unequal crossing-over: Hb Lepore (Fusion of β & δ genes)

e. Chain termination: Hb constant spring (sense mutation ⟶ α-chain with extra 31 aa)

B) **Quantitative** [↓↓ formation of α or β chains]

Hb A is *absent* in β^0 thalassemia & *decreased* in β^+ thalassemia

a. β Thalassemia [↓↓ formation of β chains]

β^0= absent β-chain synthesis β^+= reduced β-chain synthesis

- Homozygous ***(Thalassemia major)***: [$\beta^0\ \beta^0$ or $\beta^+\ \beta^+$] HbF >70%, Normal Hb A_2
- Heterozygous ***(Thalassemia minor)***: [$\beta\ \beta^0$ or $\beta\ \beta^+$] ↑↑ Hb A_2 (3.4-7 %) &↑↑Hb F (2-6 %)

Genetics:

- ***Point mutation:*** affecting transcription, mRNA splicing or translation
- ***Deletion:*** of β gene
- ***Hb Lepore:*** Fusion between β and δ genes (unequal crossing-over)

b. α Thalassemia [↓↓ formation of α chains]

Genetics:

- ***Deletion:*** of one or more of the 4 α genes (silent carrier, trait, HbH, hydrops fetalis)
- ***Chain terminator defect:*** sense mutation (Hb Constant Spring)

Syndrome	# Gene	Genotype	Hematological	C/P	Hb
α-Silent carrier	1	- α/ α α	Normal	Normal	Neonate: Hb Barts (1-2 %)
α-Thalassemia trait	2	- α/- α or --/ α α	Microcytosis Hypochromia	Normal Mild anemia	Neonate: Hb Barts (5-10 %)
HbH disease	3	- α/ - -		Mild H. anemia	Neonate: Hb Barts (20-30 %)
Hydrops fetalis	4	- -/ - -	Anisocytosis Poikilocytosis	Death IU or Early neonatal	Neonate: Hb Barts (80-90 %)

HbH disease: mild to moderate hemolytic anemia + Splenomegaly + Jaundice
Adults have HbH (β_4).Transfusion is usually not required

c. Hereditary persistence of fetal Hb (HPFH): failure of switch from γ to β chain commonly caused by deletion of β and δ genes

Genetic Control of Blood Groups

1. Major Blood Groups (ABO system)

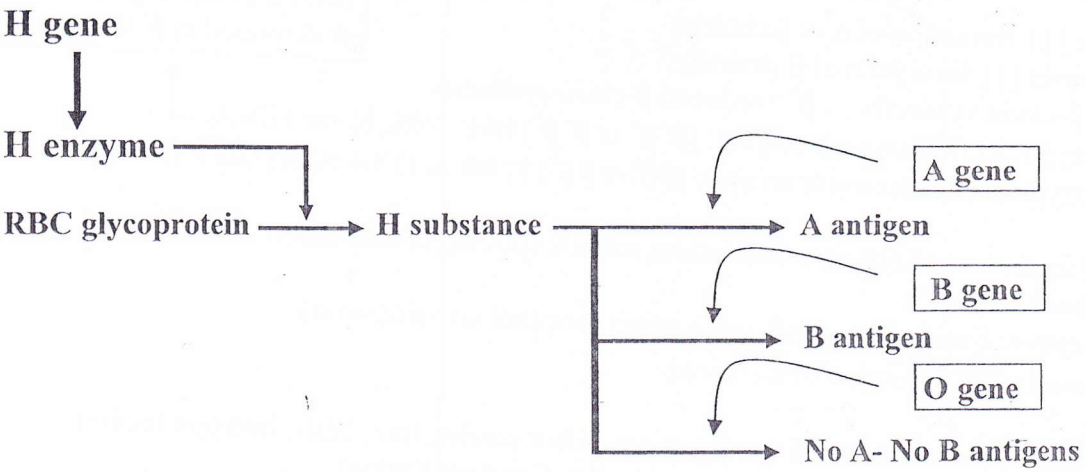
- ABO locus: One locus with 4 different alleles (A_1, A_2, B, O) on chromosome 9
- A_1 is dominant over A_2
- O is recessive
- A & B are codominant
- Antibodies (agglutinins) against ABO system are naturally occurring & of IgM type

Codominance = Both alleles are expressed in the heterozygous

Phenotype	Genotype		Antigens	Antibodies	Frequency (UK)
A	Homo-	A_1A_1, A_1A_2, A_2A_2	A	Anti-B	45
	Hetero-	A_1O, A_2O			
B	Homo-	BB	B	Anti-A	8
	Hetero-	BO			
O	OO		-	Anti-A & Anti-B	44
AB	AB		A, B	-	3

Biosynthesis of ABO Antigens:

- ABO antigens are formed from H substance be enzymes coded by A or B genes. O gene is completely inactive (leaves H substance unaltered)
- H substance is formed from a glycoprotein precursor by the H enzyme encoded by H gene
- H gene is dominant over h gene
- Bombay phenotype: Individuals with homozygous (hh) → No H enzyme → No H substance → No formation of A or B antigens (This person will have O blood group irrespective to his genotype)



Secretion of Blood group substances:

- ABO antigens are secreted in body fluid (saliva, tears, breast milk…)
- Secretion depends on Se gene
- Se gene is dominant over se gene
- Secretors are either homozygous (Se/Se) or heterozygous (Se/se)
- Non-secretors are homozygous (se/se)

Anti-D Abs occur with sensitization:
1. Blood transfusion
2. Pregnancy, abortion or delivery

2. Rh Blood Group system

- The Rh system is controlled by 3 pairs of alleles, the most important is D & d alleles
- D allele is dominant over d (DD & Dd are Rh positive)
- Other alleles are C, c, E, e
- Antibodies against Rh system *(Anti-D Antibodies)* are Not naturally occurring & of IgG type

3. Other Blood Group systems

- Kell system
- Kidd system
- Duffy system
- Lewis system

Hemolytic Disease of the newborn *(see Neonatology)*

1. Rh incompatibility
2. ABO incompatibility
3. Minor blood group incompatibility (Kell, Kidd, Duffy)

Genetic Background of Inborn Errors of Metabolism

A) Enzyme Defects

Enzymes: Biological catalysts. Virtually all enzymes are proteins (either simple or conjugated). Conjugated enzyme (holoenzyme) = Apoenzyme + Coenzyme

Apoenzyme: It is the protein part of the holoenzyme

Coenzyme: It is the organic non-protein part of the holoenzyme (e.g., vitamin…)

- ☒ Enzyme defect may be:
  - Genetically determined
  - New mutation
- ☒ This may lead to:
  - ↓↓ Enzyme synthesis
  - ↓↓ Enzyme activity due to: Distortion of enzyme configuration
    ↓↓ Affinity of the apoenzyme to substrate or coenzyme
- ☒ Enzyme defect leads to metabolic block:

a. Accumulation of precursors

Disease		Enzyme Defect	Accumulated substance
Galactosemia		Galactose-1-P-uridyltransferase	Galactose & Galactitol
GSD *(Von Gierke)*		Glucose 6-Phosphatase	Glycogen
Gaucher		Glucocerebrosidase (β-Glucosidase)	Glucocerebrosides (glycolipid)
Niemann-Pick		Sphingomyelinase	Sphingomyeline
MPS *(Hurler)*		α-L- Iduronidase	GAG = Glycosaminoglycan
GM_1 Gangliosidosis		β-Galactosidase	Gangliosides
GM_2	**Tay-Sachs**	Hexosaminidase A	Gangliosides
	Sandhoff	Hexosaminidase A & B	Gangliosides

b. Deficiency of end-product

Albinism: (↓↓ Melanin) Tyrosine —(*Tyrosinase*)→ Melanin

c. Opening of alternative pathway

Normally: Phenylalanine —(*P.hydroxylase*)→ Tyrosine

Phenylketonuria: ↑↑ Phenylalanine → ↑↑ Phenylpyruvate, lactate& acetate

B) Transport across cell membranes

a. Transport across cell membrane

Specific Vit. B_{12} malabsorption due defective receptors for IF-B_{12} complex

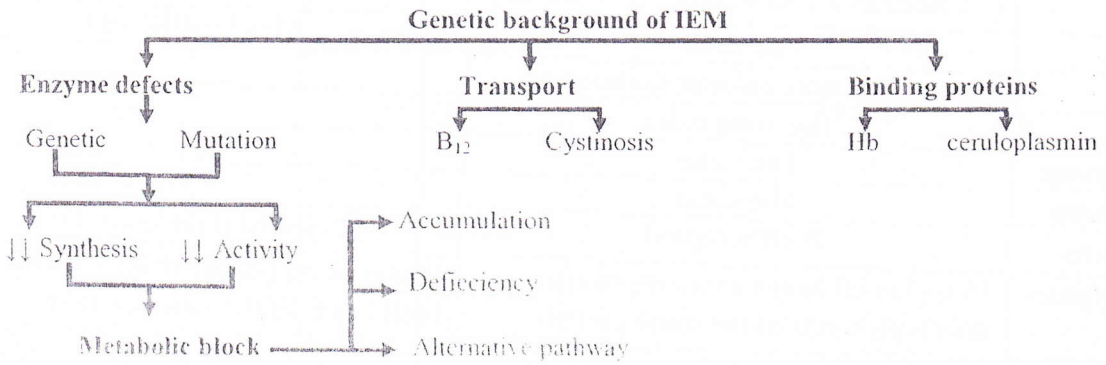
b. Transport across lysosomal membrane

Cystinosis: Trapping of cystine inside the lysosomes. [Action of Cysteamine??]

C) Binding Proteins

a. Hemoglobin carries O_2: HbM cannot carry O_2

b. Ceruloplasmin carries Copper: Wilson disease



Twin Studies

(Multiple Pregnancies)

Incidence

- Twins = 1: 86 Triplets = 1: 86^2 Quadruplets = 1: 86^3
- 1/3 of twins are monozygotic (MZ) = Uniovular = Identical
- 2/3 of twins are dizygotic (DZ) = Binovular = Non-identical

Chorion is formed around D_3
Amnion is formed around D_8

Etiology

☒ Monozygotic twins: develop from splitting of a single zygote (single fertilized ovum)

Splitting	Freq.	Placenta	Chorion	Amnion	Cords	Term
1st 3 days	25 %	2	2	2	2	Dichorionic-diamniotic
Days 4-8*	75 %	1	1	2	2	Monochorionic-diamniotic
Days 9-15	-	1	1	1	2	Monorionic-monoamniotic
> D 15	-	1	1	1	2	Conjoined twins

Conjoined twins (Craniopagus, thoracopagus…)

☒ Dizygotic twins: develop from 2 fertilized ova [2 ova + 2 sperms]
Always Dichorionic-Diamniotic

Definitions

Concordance: Both members have the same trait
Discordance: Only one co-twin has the trait (i.e., Different from his partner)
Monozygotic twins: have the same genotype (one fertilized ovum)
So any difference in phenotype must be due to environmental factors
Dizygotic twins: have different genotypes (two fertilized ova)
So any difference in phenotype may be due to environmental or genetic factors

Genetic Applications

- If the concordance rate of a specifics disease in MZT is > DZT ⟶ genetic factors
- If the concordance rate of a specifics disease in DZT of the same sex is > DZT of different sex ⟶ sex effect
- Concordance rate for type 2 DM in MZT is almost 100%
- Concordance rate for type 1 DM in MZT is 30-50%
- Concordance rate for DM in MZT is > DZT
- Concordance rate for CF in MZT is almost 100% *(purely genetic)*
- Concordance rate for SLE in MZT is 50% & 5% in DZT *(Not only genetic)*

Genetic component is stronger in type 2 DM

Type		MZT	DZT
Etiology		Splitting of a single zygote (One ovum)	Develop from 2 fertilized ova
Placenta & Fetal membrane		▪ 75% are (Monochorionic-diamniotic): [1 Placenta + 1 Chorion + 2 Amnions] ▪ 25% are (Dichorionic-diamniotic): [2 Placentas + 2 Chorions + 2 Amnions]	Always Dichorionic-diamniotic [2 Placentas + 2 Chorions + 2 Amnions]
Markers	**Sex**	The same	May be different
	Hair	The same color & texture	"
	Eye	The same color	"
	Blood group	The same	"
	HLA group	The same	"
Skin grafts		Well accepted	Rejected if different HLA
Dermatoglyphics		Homolateral hands are more similar than both hands of the same co-twin	Homolateral hands are less similar than both hands of the same co-twin

Genetic Counseling

Definition *(Talking to the family)*

It is a **communication** process offered to high risk or affected individual to understand the nature of a genetic disease, its transmission & the options available for management
It should be neutral & non-directive. The counselor should provide support to the family to cope with decisions taken

Requirements

a. Accurate **family history** & **pedigree** (including all relatives, abortions…)
b. Full confirmed **diagnosis**
c. Reviewing the updated medical, laboratory & genetic **information** about the disorder including the mode of inheritance & RR

Indications of Genetic Counseling

Common situations of Genetic Counseling:
- ☒ Prenatal diagnosis [? Abortion]
- ☒ Newborn with congenital anomalies [? Support]
- ☒ Later in life [? Premarital ? Prenatal]

1. Advance parental age
   - ☒ Maternal age > 35 yrs
   - ☒ Paternal age > 50 yrs
2. Consanguinity
3. Spontaneous abortion
4. Congenital malformation & dysmorphology
5. Developmental delay & mental retardation
6. Atypical (ambiguous) genitalia
7. Disorders of sex development (complete androgen insensitivity…)
8. Short stature (♀)
9. Primary amenorrhea
10. Infertility
11. +Ve Antenatal screening tests
    - ☒ +Ve maternal serum screening test (e.g., triple test)
    - ☒ +Ve US screening test
    - ☒ Fetal karyotype & sex??
12. Teratogen exposure (Drug, substance or exposure…)
13. Heterozygote screening based on ethnic risk (Carrier detection)
    - ☒ Sickle cell anemia [Africa]
    - ☒ Tay-Sachs, Gaucher, Canavan [Jewish]
    - ☒ Thalassemia [Mediterranean]
14. Adult-onset genetic disease
    - ☒ Huntington chorea
    - ☒ Malignancy: (Breast, ovarian, colon…)

Recurrence Risk

A) Single gene inheritance: *(Give examples on AD, AR, Sex-linked)*
B) Multifactorial inheritance: 2-4 %

Diagnostic problems

A) Heterogeneity: The same C/P is caused by affection of more than one gene (Each one can cause the disease), with *different* modes of inheritance. Examples: Homocystinuria, MPS
B) Phenocopies: Conditions similar to genetic diseases but they are caused by environmental factors [e.g., Microcephaly…]
C) Sporadic cases *(Common in modern families)*. The disorder may be:
   - ☒ Non-genetic
   - ☒ Chromosomal (↓ RR = 1 %)
   - ☒ Multifactorial (RR = 2-4%)
   - ☒ AR (normal parents) "RR = 25%"
   - ☒ AD (new mutation) "RR = 1%"
   - ☒ XLR (new mutation or carrier mother)

Dysmorphism

Definition

It is a morphological developmental abnormality of **prenatal** onset

2-4% of newborn babies have a physical anomaly

Classification

A) Single primary defects*: More common, usually multifactorial

B) Multiple malformation syndromes: One or more developmental abnormalities affecting 2 or more systems. It is usually due to chromosomal abnormalities or teratogenic exposure

Single primary defects

A) Malformation

- Localized error in morphogenesis
- Examples: CHD (RR = 2-5%), polydactyly, CL ± P

Fetal movement is an important factor in the development of the M.Sk.System

B) Deformation

- Alteration in the shape or structure of a normally differentiated organ
- It usually affects the musculoskeletal system (e.g., Talipes…)
- It is due to ↓↓ fetal movement

a. Intrinsic factors (Fetal neuromuscular apparatus):

- CNS: developmental defects
- Motor unit: Muscles: Congenital myopathy (nemaline rod), Myotonic dystrophy
  Nerves: SMA type 1 (Werding-Hoffmann)

b. Extrinsic factors:

- Oligohydramnios
- Multiple pregnancies
- Malpresentation (Breech)
- Uterine shape: Bicornuate or septate uterus
- Uterine tumors: Fibroid

C) Disruption

- Destruction of a previously normal part
- Destruction may be mechanical, vascular, infectious
  - **Mechanical:** Amniotic bands ⟶ Amputation of a digit
  - **Vascular:** Interruption of blood supply leads to ischemic infarction
    Examples: Mesenteric ⟶ Intestinal atresia & CNS ⟶ Porencephaly (brain cyst)
  - **Infectious:** TORCH infection

D) Sequence

- **Single** primary defect results in a cascade of subsequent events
- Although there are multiple anomalies, it is considered as a single primary defect
- Example: Pierre-Robin syndrome
  The single primary defect is mandibular hypoplasia ⟶
  Dropping of the tongue backwards ⟶ "U-shaped" Cleft palate

o Syndrome

- Multiple malformations that occur together due to a single known underlying cause
- Example: Down syndrome

o Association

- Multiple malformations that occur together in a **non-random** manner, usually due to unknown cause
- Example: VATER/VACTERL association

VATER/VACTERL:
Vertebral, Anorectal, Cardiac, Trachea, Esophagus, Renal, Limb

Teratogens

Definition

Teratogen is any **environmental** agent (drug, substance or exposure) that interferes with normal **embryonic** development (Structure, growth or function)

Examples:

- Drugs: Alcohol, captopril, cocaine, indomethacin, lithium, phenytoin, propranolol, streptomycin, tetracycline, thalidomide (phocomelia), warfarin… *(See Neonatology)*
- Infection
- Maternal DM & PKU
- Radiation (ionizing & non-ionizing)

Mechanism of teratogens:
1. DNA damage
2. Cell death
3. Vascular insult
4. Delayed differentiation

Effect

The effect depends on:

☒ Nature of the teratogen *(The mechanism is usually unknown)*
Warfarin is teratogenic on fetal cartilages [# carboxylation of glutamic acid]

☒ Time of exposure (fetal age)

a. Weeks 1-3 (embryonic stage): All or none fashion; either killing or no effect
b. Weeks 3-10 (organogenesis): organs are most susceptible to damage
c. Weeks 10-40 (fetal growth& maturation): ↓↓ risk but may interfere with function

☒ Genetic predisposition: teratogens are not universal "Pharmacogenetics"

Prevention

- Avoid drugs, infection & radiation
- Control of maternal DM, PKU
- Abortion

Pharmacogenetics

Definition

It is the genetically determined variations in drug metabolism & response
Pharmacogenetic variability is the cause of the observed wide range of drug response
Examples:

- Exaggerated physiologic effect
- Drug resistance
- Drug side effects

Pharmacogenetics will help to identify "The right drug for the right patient"

Half-lives of several drugs are more similar in MZT > DZT "Genetic factors"

Factors affecting the response to drugs

☒ Genetic factors "Pharmacogenetics"
☒ HLA typing
☒ Physiologic factors (age, sex, pregnancy)
Chloramphenicol (usual dose) ⟶ **Gray baby syndrome** in *premature & newborn* infants
☒ Environmental factors (Diet, smoking)
☒ Route of administration
☒ Organ failure (Liver & Kidney)
☒ Drug interaction

- Valproate ⟶ ↑↑ toxicity of Phenytoin, Phenobarbital & Lamotrigene
- Diuretics ⟶ ↑↑ Digitalis toxicity
- Aminoglycosides + frusemide (vancomycin & amphotericin B) ⟶↑↑ Nephrotoxicity
- Antacids ⟶ ↓↓ Absorption of steroids, NSAIDs & iron

☒ Patient compliance

Drug metabolism (Biotransformation)
Phase I (Oxidation, Reduction, Hydrolysis)
Phase II (Conjugation) with:

- Glucuronic acid: Chloramphenicol, Paracetamol
- Acetic acid: Isoniazide, sulfonamides
- Glycine: aspirin

A) Effects of genes on drug metabolism

1. Acetylation

Individuals homozygous for "slow acetylation" polymorphism are more susceptible to:

- Isoniazide (INH) toxicity "peripheral neuritis"
- Sulfonamides induced Stevens-Johnson syndrome

2. Polymorphism in Cytochrome P_{450}

CYPs are heme-containing enzymes

- CYPs are the most important enzymes of phase I biotransformation
- Fetal expression is limited, functional activity is acquired postnatally (4 months- 4 years)
- Enzyme activity can be induced or inhibited be various agents
- They are grouped into:

  Families (I, 2, 3…) ⟶ Subfamilies (A, B, C…) ⟶ Members (1, 2, 3…)
- CYPs genes are highly polymorphic (↑↑ alleles) ⟶ Individual variation in drug metabolism

	Drug substrates	Inducers	Inhibitors
CYP_1A_2 (4 months)	▪ Acetaminophen ▪ Theophylline ▪ Caffeine	▪ Cigarette smoke	▪ Cimetidine
CYP_2D_6 (4 years)	▪ Codeine ▪ Captopril ▪ Haloperidol ▪ Propranolol	--	▪ Cimetidine ▪ Quinidine
CYP_3A_4 (1 year)	▪ Acetaminophen, Theophylline ▪ Cyclophosphamide, cyclosporine ▪ Erythromycin ▪ Verapamil	▪ Phenytoin ▪ Phenobarbitone ▪ Rifampicin	▪ Erythromycin ▪ Fluconazole

3. Alcohol dehydrogenase polymorphism: Individual variation in response to ethanol

4. HLA type: ↑↑ Some drug toxicity in certain HLA types (↑↑ gold toxicity in HLA-DRW_3)

B) Effects of drugs on certain genetic diseases

Agents precipitating hemolysis in G6PD deficiency:
2. Fava beans
3. Antibiotics: sulfonamides, chloramphenicol, nitrofurantoin
4. Antimalarial: primaquine
5. Aspirin, Vit K
6. Chemicals: Benzene, naphthaline

1. G6PD Deficiency (XL-R)

There are > 100 enzyme variants of G6PD

G-6-PD B^+ is the normal enzyme

G-6-PD A^+ is a normal variant

G-6-PD B^- (5-40 % activity)

G-6-PD A^- (5-15 % activity)] Abnormal variants

Hemolysis occurs in patients with G-6-PD deficiency on exposure to certain drugs (oxidant stress)

2. Malignant hyperthermia

AD condition due to mutation of Ca ion release channels

C/P: Malignant hyperthermia in response to halothane or succinylcholine

3. Pseudocholinestrase (serum cholinestrase)

Individuals with ↓↓ enzyme activity develop prolonged paralysis in response to succinylcholine *(succinylcholine apnea)*

Types of cholinesterase enzymes:
True CE: Endogenous A.Ch.
Pseudo CE: Exogenous A.Ch.
Succinyl choline

4. Acatalasia

Individuals with absent catalse enzyme activity [$H_2O_2 \longrightarrow H_2O + O_2$]

develop methemoglobinemia in response to oxidant stresses (Due to accumulation of H_2O_2)

Gene Therapy

Definition

It is transfer of recombinant DNA into human cells for correction of a disease

Requirements

1. **Preparation of gene** *(How? See Recombinant DNA)*
2. **Vector (viral* or non-viral):** It is a DNA molecule used to carry DNA region of interest
3. **Human cell:** transfer & integration of the gene can be directed to:
   - ☒ Somatic cell: Not heritable
   - ☒ Germ cell: Heritable to successive generations (Ethically not accepted)

Principle

1. Gene is attached to the vector
2. Tansfection of the human cell by the vector
   - ☒ Ex vivo gene delivery: Cells are taken from the patient, gene is inserted& returned back
   - ☒ In vivo gene delivery: Direct transfection of the human cell (e.g., portal v for liver cells)
3. The delivered gene is incorporated into the nucleus
4. Transcription of the delivered gene into mRNA
5. Translation into the deficient protein "Therapy"
6. The formed protein may act in the target cell or secreted to exert effect in distant cells

Fate of the delivered gene

1. Degradation by lysosomes: No effect
2. Permanent expression: Gene is integrated into the human DNA, so transmitted to daughter cells
3. Transient expression : Maintained in the nucleus as an episome, so it is active in such cell only but not transmitted to daughter cells

Applications of Gene Therapy

Gene therapy remains experimental

- ☒ Treatment of genetic diseases (ADA deficiency…)
- ☒ Treatment of acquired diseases (Infectious diseases "HIV", malignancy…)

Vector Systems

Ideal Vector

- Reaches the target cells by **in vivo administration**
- **Single** administration
- **Integrated safely** in the genome (without disturbing normal genes)
- Not integrated in **oncogenic** sites
- Removes the **defective** gene & replaces it with normal gene
- **Transmitted** to daughter cells "permanent expression"

Classification

A) Viral vectors
1. Retrovirus
2. Adenovirus
3. Adeno-associated virus

B) Non-viral vectors
1. Naked (Plasmid)
2. Liposomes
3. Ligands

Risks of Viral Vectors *(All viral vectors)*

- Insertional mutations (random integration into normal genes)
- Infectious complications due to activation of the recombinant virus into the wild form
- Inflammatory & Immune responses

A) Viral vectors *(Theses viruses require genetic modification of the wild form)*

1. Retrovirus

a. Wild virus: Replication occurs as follow

- ☒ Attachment through specific receptors (CD_4 on T_H cells in cases of HIV infection)
- ☒ Endocytosis & Eclipse
- ☒ Reverse transcription

Viral RNA ⟶ DNA (single strand) ⟶ ds DNA ⟶ integrated into human DNA
(Reverse transcriptase)

- ☒ Transcription into mRNA
- ☒ Translation & production of viral proteins (Viral capsid & enzymes)
- ☒ Capsidation (assembly)
- ☒ Release

Viral Genome contains the following genes:
1. GAG gene: codes for capsid proteins
2. POL: codes for reverse transcriptase
3. ENV: codes for envelope proteins
4. Psi ψ sequence: important for packaging events
5. LTR (Long terminal repeats): powerful promoters

Packaging = Integration + formation of viral partic..s

b. Recombinant virus

- ☒ Recombinant virus contains:
  1. Psi ψ sequence
  2. LTR
  3. Therapeutic gene *replacing* GAG, POL, ENV (So it is a *defective* virus)

- ☒ GAG, POL& ENV genes from a helper virus (No Psi ψ) are permanently integrated in the genome of packaging cell lines

- ☒ The recombinant virus is transfected into the packaging cell
- ☒ The recombinant virus uses proteins encoded by the packaging cell genome to produce viral particles which contain only therapeutic gene (No GAG, POL & ENV genes).
- ☒ Theses new viral particles are not infectious
- ☒ Transfection of target human cell by the recombinant virus *(How? 2 methods)*
- ☒ Integration of the therapeutic gene *(Mention the fate?)*

2. Adenovirus & adenoassociated virus

Type	Advantages	Limitations
Retrovirus Small virus RNA virus	▪ Permanent expression (Integration) ▪ Transmitted to daughter cells	▪ Only small gene can be used (9 Kb) ▪ Integration depends on cell division ▪ Ineffective in resting cells
Adenovirus Big virus DNA virus	▪ Large gene can be used (39 Kb) ▪ Effective in resting cells (non-dividing) ▪ Effective in Rx of acquired condition (malignancy) ▪ Effective in Rx of genetic respiratory diseases (CF). Tendency to infection of cells lining airways	▪ Transient expression (No Integration) ▪ No transmission to daughter cells ▪ Episomal particle ▪ Regular multiple administration ▪ Inflammatory reaction
Adeno-associated virus Defective virus DNA virus	▪ Effective in resting cells (non-dividing) ▪ Wild virus has site specific integration on chromosome 19 (not recombinant one)	▪ Only small gene can be used (< 5 Kb) ▪ Transient expression (No Integration) ▪ It is defective virus (coinfection with adenovirus)

Mention risks of viral vectors (I I I)

B) Non-Viral vectors

1. Naked DNA (Plasmid DNA)

Small, extra-chromosomal, circular dsDNA molecules found in bacteria

Advantages:

- Easy production in large amount
- Large gene can be used (10 Kb)

2. Liposomes

- Incorporation of plasmid into small lipid vesicle → ↑↑ delivery to target cell
- Liposomes fuse with cell membrane of target cell → Internalization
- Lysosomal digestion of lipid & release of DNA into the nucleus
- The recombinant DNA is maintained as episome (Transient expression)

3. Ligands

- Linkage of plasmid to a protein conjugate
- The protein helps to direct the plasmid DNA to specific cell containing specific receptors

Immune Reaction against vectors

☒ Cell mediated immunity: Cytotoxic T cells react against vector& formed gene product

☒ Humoral immunity: Neutralizing Abs against the vector (# subsequent transfection)

Alternative strategies for gene therapy

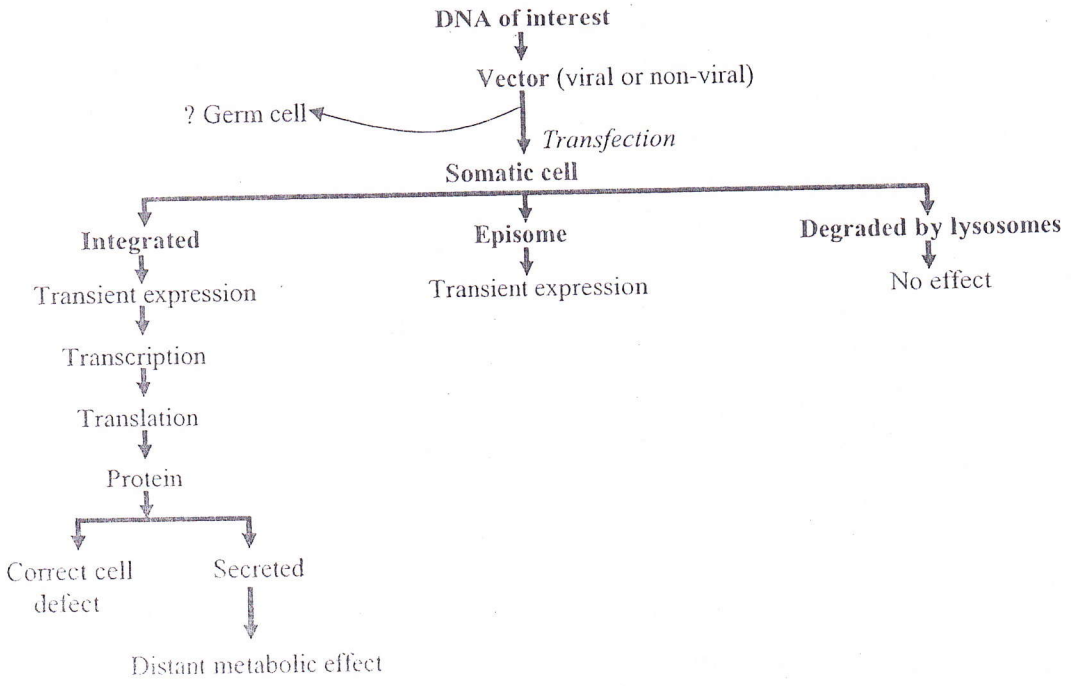
Organoids: Ex vivo genetically altered cells to produce specific gene product

These cells are returned back to the patient& placed SC, in BV, peritoneal cavity

Produce Factor VIII (Hemophilia), β-Glucuronidase (Type VII MPS)…

Diseases targets for Gene Therapy

- Gene therapy remains experimental & challenging
- Many common diseases, such as HTN& DM are caused by combined effect of many genes and so are not candidate for gene therapy *(at least now)*
- The first human gene therapy trial was in ADA deficiency in 1990 (relatively small gene)



Prevention of Genetic Diseases

A) Epidemiology

- Genetic registry & counseling (Carrier detection)
- Identification of mutagens

B) Prenatal Diagnosis

1. Maternal serum α-fetoprotein (MSAFP)

- ☒ ↓↓ MSAFP: Down syndrome & other trisomies
- ☒ ↑↑ MSAFP: Neural tube defects (anencephaly, encephalocele, spina bifida)
  Hydrocephalus
  GIT: TOF, Intestinal atresia
  Renal: Congenital nephrosis, Obstructive uropathy
  Twins, IUFD

2. Fetal US

- ☒ Assessment of fetal growth, gestational age& well-being
- ☒ Nuchal Translucency thickening (NT): thickening of the fat pad at the back of the neck
- ☒ Dilated cerebral ventricular system (hydrocephalus)
- ☒ Dilated renal pelvicalyceal system [large UB in PUV]
- ☒ Absent stomach (TOF), Double-bubble (duodenal atresia), Distended loops (IO)
- ☒ Fetal echocardiography

3. Amniocetesis & Chorionic Villus Sample (CVS)

	Amniocentesis	Chorionic villus sample
Timing	2nd Trimester (14-16 weeks of gestation)	1st Trimester (9-12 weeks of gestation)
Anesthesia	LA or GA	LA or GA
Technique	Transabdominal sample of amniotic fluid (US guided)	Transvaginal or Transabdominal biopsy of chorionic villi (US guided)
Obtained Cells	**1. Fetal sexing** (XL diseases e.g., hemophilia, Duchenne …) **2. Karyotyping** (chromosomal abnormalities e.g., Down, trisomies…) **3. DNA analysis** (Thalassemia, Sickle cell disease, cystic fibrosis…) **4. Enzyme assay** (Galactosemia, GSD, Gaucher, Niemann-Pick, MPS, GM_1, GM_2)	
Liquid phase (in amniocetesis)	1. α-fetoprotein (causes as MSAFP) 2. Bilirubin (erythroblastosis fetalis) 3. Lung maturity (L/S ratio…) 4. Renal maturity (creatinine) 5. CAH (↑↑ Ketosteroids) 6. Congenital hypothyroidism (↓↓ T_4)	??
Complications	▪ Abortion ▪ Fetal injury ▪ Hemorrhage ▪ Rh sensitization ▪ Infection (amnionitis)	▪ Abortion (2% higher) ▪ Amnion puncture ▪ Hemorrhage ▪ Rh sensitization ▪ Infection
Pros & Cons	▪ Less risk of abortion ▪ Technically easier ▪ ↓↓ yield of cells (cells must be cultured)	▪ ↑↑ yield of cells (rapid diagnosis) ▪ Early in pregnancy (when termination is less risky& less emotionally traumatic)

4. Fetoscopy& Fetal Tissue Sampling

Transabdominal introduction of fetoscope under LA (US guided). 2nd trimester

a. Direct visualization (structural anomalies e.g., phocomelia, neural tube defects…)
b. Cordocentesis (Fetal blood sampling)
   - Hemoglobinopathies: Sickle cell anemia
   - Coagulation disorders (Hemophilia)
   - Neonatal alloimmune thrombocytopenia
   - Fetal infection (Toxoplasmosis)
   - Immunodeficiency
   - Karyotyping, DNA analysis & enzyme assay
c. Fetal liver biopsy (PKU & OTC deficiency)
d. Fetal skin biopsy (Epidermolysis bullosa)

OTC = Ornithine transcarbamoylase
Most common type of urea cycle defects
XL-R الوحيد

C) Pre-implantation genetic diagnosis (PGD)

Definition

Diagnosis of genetic diseases before pregnancy is established
Must be preceded by IVF & ICSI

Blastomeres: Cells formed by cleavage of the zygote. Each one contains the full genetic material of the baby

Technique

- ☒ Stimulation of maternal ovulation ( )
- ☒ Ovum retrieval
- ☒ Fertilization by sperm (Intracytoplamic sperm injection)
- ☒ Embryo is cultured till the stage of 6-8 blastomeres
- ☒ Single blastomere is separated from the embryo (micromanipulation technique)
- ☒ Genetic make-up is determined [Each blastomere contains the full genetic material]
- ☒ The remaining blastomeres are left intact & allowed to grow (if no disease)
- ☒ Transfer to mother's uterus (Usually 3 non-affected embryos are transferred)

Methods of Diagnosis

a. Karyotyping (important in XL-R diseases when the exact mutation is unknown)
   Only ♀ zygotes are transferred to the mother's uterus
b. PCR & ARMS
   - Detection of a specific disease
   - Amplification of DNA followed by analysis e.g., DNA sequencing
c. FISH:

Complete chromosomes
Chromosomal subregions
Microdeletion syndromes

Advantages of PGD

a. Very early (!! Before pregnancy, so avoids emotional trauma)
b. Couples who are carriers of AR genetic diseases can have healthy children

Diseases which can be detected by PGD

- ☒ Chromosomal: Trisomies (21, 18, 13), Turner, Klinefelter
- ☒ AR: Cystic fibrosis, Tay-Sachs, (? Gaucher)
- ☒ XL-R: Hemophilia, Duchenne, Fragile X

D) Screening of Genetic Diseases

1. **Neonatal screening:** Congenital hypothyroidism, PKU, Galactosemia, Sickle, CF, MC
2. **Heterozygote screening** based on ethnic risk (Carrier detection)
   - ☒ Sickle cell anemia [Africa]
   - ☒ Tay-Sachs, Gaucher, Canavan [Jewish]
   - ☒ Thalassemia [Mediterranean]
3. **Carrier detection** in relatives of affected individuals
4. **Screening for at risk population** (Screening for hyperlipidemia to detect persons at risk of ischemic heart disease)

Treatment of Genetic Diseases

1. Gene therapy *(Give examples)*

2. Enzyme Induction

Phenobarbitone in Crigler-Najjar syndrome type II (AD)

3. Enzyme Replacement

- ☒ Gaucher disease
- ☒ Pompe disease (GSD type II)
- ☒ Fabry disease
- ☒ ADA deficiency
- ☒ Some MPS
- ☒ Cystic fibrosis

4. Recombinant proteins

- ☒ GH
- ☒ Insulin
- ☒ Factor VIII
- ☒ EPO
- ☒ GM-CSF
- ☒ Interferon

5. Replacement of Hormones

- ☒ Hydrocortisone (CAH)
- ☒ 9-α fludrocortisol (CAH)
- ☒ GH (Hypopituitarism)
- ☒ Thyroxine (Congenital hypothyroidism)

6. Replacement of vitamins

- ☒ B_1 (Maple syrup urine disease)
- ☒ B_6 (Homocystinuria)
- ☒ B_{12} (Methylmalonic acidemia)
- ☒ Biotin (Propionic acidemia)
- ☒ Folic acid (Megaloblastic anemia)
- ☒ Vitamin D (Vitamin D resistant rickets)

7. Dietary restriction

- ☒ Maple syrup urine (V I L)
- ☒ Methionine (Homocystinuria)
- ☒ PKU (Phenylalanine)
- ☒ Urea cycle disease (proteins)
- ☒ Galactosemia (galactose, Lactose)
- ☒ Hypercholesterolemia (Lipids)

8. Induction of alternative pathways

Na benzoate in urea cycle defects (to eliminate NH_3)

9. Preventive therapy

Avoidance of certain drugs in G6PD deficiency

10. Removal of abnormal tissue

- Splenectomy (Hereditary spherocytosis)
- Colectomy (Polyposis coli)

11. Transplantation

Renal transplantation for management of polycystic kidneys (PKD)

12. Portocaval anastomosis

In cases of portal hypertension (GSD type IV)

13. Extracorporeal therapy

Plasmapheresis in the Rx of hypercholesterolemia

Polymorphism& Genetic Markers

People are genetically very similar (99.9% identical)

Polymorphism

- Genetic differences between individuals which provides variation within a species
- The occurrence of 2 or more alleles at a locus in a frequency greater than that can be maintained by mutation alone
- Polymorphisms are more common in the non-coding regions of DNA
- Polymorphisms in the coding regions of DNA are responsible for:
  - ☒ Variation in blood groups
  - ☒ Variation in HLA typing
  - ☒ Variation in drug metabolism (pharmacogenetics)
  - ☒ Variation in enzyme activity (There are > 100 enzyme variants of G6PD enzyme)

Clinical Importance of Polymorphism

Most polymorphisms produce no clinical phenotype

1. **Blood grouping& Tissue typing (HLA)**
2. **Pharmacogenetics**
3. **Forensic medicine**
4. **Problems with identity, zygosity, paternity** (DNA finger printing)
5. **Genetic markers** (Linkage studies)
6. **Some diseases occur in a polymorphic frequencies**
   - ☒ Sickle cell anemia
   - ☒ Thalassemia

   } Heterozygous shows mild manifestations while homozygous is severe

Forms& Diagnosis of Polymorphism

A) **DNA technology**
1. **RFLP:** When variations of DNA sequence involve restriction sites
   Detected by Southern blotting technique
2. **PCR**
3. **DNA sequencing**
4. **Single nucleotide polymorphism (SNP):** Different people have a different nucleotide at a given location on a chromosome
5. **VNTR** (Variable numbers of tandem repeats):

Pre-axial: Radial or tibial
Post-axial: Ulnar or fibular

B) **Altered gene products**
1. Variation in blood groups & HLA typing
2. Variation in enzyme activity due to altered protein structure (altered activity, thermostability or electrophoretic properties)

C) **Physical features:** Post-axial polydactyly
D) **Chromosomal heteromorphism** (= Structural polymorphism in chromosomes)
1. Variation in the size of Y chromosome
2. Variation in the fragile sites

Repeated sequences in Human DNA

	Repeat size (bp)	Total size (kb)	Features
Satellite	5-200	100	
Minisatellite	10-60	20	2 families: VNTR & telomeric family
Microsatellite	1-4	1	Repeats of A, CA

(VNTR = Variable numbers of tandem repeats)

Genetic Marker

It is a simple inherited genetic trait (e.g., DNA polymorphism*, biochemical marker, blood group…) that can be linked to a disease locus, so can be used for diagnosis & gene mapping

Linkage

The co-segregation of 2 non-allelic genes which have their loci very close to each other on the same chromosome & so they move together during meiosis

Linkage analysis (Indirect DNA Diagnosis)

- The use of a genetic marker (e.g., DNA polymorphism…) to identify a specific gene
- It requires a family with more than one affected member
- Markers that are not associated with the disease are randomly shared between affected & unaffected members of the family
- Markers that are closely linked to the affected gene are found much more common in the affected members (diseased)

Genetic markers that are usually used are:
1. SNPs
2. Microsatellites

Clinical Importance of Linkage

1. **Determination of the genotype**
2. **Determination of the mode of inheritance**
3. **Gene mapping**
4. **Prenatal diagnosis**
5. **Presymptomatic diagnosis**
6. **Carrier detection**
7. **Determination of genetic factors in complex traits** (e.g., DM)

Gene mapping

Definition

Assignment of genes to specific loci

Methods

1. Linkage studies
   - It requires a family with more than one affected member (Family study)
   - It is a measure of genetic distance
2. Somatic cell genetic method
   - Cells lacking specific chromosomal segment lack a specific gene
   - Cells having specific chromosomal segment show a specific gene
3. Cytogenetic study
   FISH: directly visualizes the specific gene
4. Gene dosage studies
   - Amount of gene product (protein) is directly proportionate to the number of copies
   - Normal = amount of protein
   - Trisomy = 1½ amount of protein
   - Monosomy = ½ amount of protein

Importance

1. Understand the anatomy of the human genome
2. Understand the function of the human genome
3. Gene therapy
4. Analysis of heterogeneity

Question 80 of 124 A. Autosomes B. B. Aneuploidy C. Triploidy D. Mosaic E. Chimera F. Trisomy G. Monosomy H. Instructions: Match the above terms with their correct description from the list below: 1. An entire half set of chromosomes 2. Two different cell lines derived from a single zygote 3. Three copies of one chromosome 4. Chromosomes 1-22 5. Two different cell lines caused by the fusion of two zygotes 6. An extra or missing chromosome	Question 81 of 124 All the following statements about mitochondrial inheritance are true except: A. Transmission occurs through the maternal line B. Sperm has negligible amount of mitochondrial DNA C. Is seen in some types of diabetes mellitus and deafness D. Both sexes may be affected E. Daughters of affected males are obligate carriers
Question 82 of 124 One of the following statements about consanguinity is incorrect: A. Is seen more commonly in certain ethnic groups B. Presence of consanguinity favours autosomal recessive (AR) inheritance C. Two brothers marrying two sisters is an example D. The rarer the disorder, the higher will be the proportion of affected individuals due to parental consanguinity E. Increases the likelihood of birth defects only slightly	Question 83 of 124 A young couple attend your clinic for genetic counselling. There is a history of phenylketonuria on the husband's side of the family and they have questions about the disorder and its inheritance. Which of the following statements about PKU is false? A. The inheritance is autosomal recessive B. PKU is classically due to deficiency of the enzyme phenylalanine hydroxylase C. Mental retardation is only apparent after a few months D. Over 50% of affected infants have EEG abnormalities E. Serum tyrosine levels are typically low
Question 84 of 124 All of the following statements about Glucose-6-phosphate dehydrogenase deficiency are false except: A. Is more common in women than in men. B. Is caused by mutations of the G6PD gene on chromosome 4. C. Often leads to chronic haemolysis. D. Requires treatment with desferrioxamine to prevent iron overload. E. Is associated with haemolytic crises induced by sulfonamides.	Question 85 of 124 The following are true of autosomal dominant (AD) inheritance pattern except: A. Both males and females are equally affected B. Achondroplasia and Marfan syndrome are common examples C. Late onset disorders are usually inherited in an AD pattern D. Both parents are usually asymptomatic carriers E. Variable expression occurs frequently

Question 86 of 124

A. 45, X
B. 47, XYY
C. 47, XXY
D. 47, XX+21
E. 47,XX+13
F. 47, XXX
G. 47, XX+18

Instructions: Match the following karyotypes with the correct phenotype from the list below:

1. Normal male with extra Y chromosome
2. Female with Down syndrome
3. Normal female with extra X chromosome
4. Male with Klinefelter syndrome
5. Female with Turner syndrome
6. Female with Patau syndrome

Question 87 of 124

A 56 year old lady brings her 15 year old son to see the GP. The boy complains of burning pains in his hands and feet that can be debilitating at times. He is noted to have a diffuse red skin rash, particularly over his trunk and back. His mother has been diagnosed with hypertrophic cardiomyopathy.

What is the most likely diagnosis?:-

A. Tay-Sachs disease
B. Fabry's disease
C. Homocysteinuria
D. Neimann-Pick disease
E. Tangier disease

Question 88 of 124

A child has had his chromosomes analysed in the course of various investigations and the result is: 46 XY, t (2;5)(q35;p21.3}.

Which of the following statements is correct?:-

A. He has more than 46 chromosomes
B. The result shows all his genetic defects
C. There is a translocation between the short arm of chromosome 2 and the short arm of chromosome 5
D. He is likely to be infertile
E. There is an increased risk of him having a child with difficulties

Question 89 of 124

An 11-year-old boy has a long history of recurrent chest infections and poor weight gain. Which of the following findings is least likely to support a diagnosis of cystic fibrosis?

A. Sweat Na concentration of 93 mmol/l
B. Sinusitis
C. Peribronchial thickening on CXR
D. A sweat Na greater than sweat Cl
E. Metabolic alkalosis

Question 90 of 124

A. Chromosome
B. Chromatid
C. Centromere
D. Autosome
E. Gene
F. Chromatin

Choose the most likely definitions for each of the above from the list given below:

1. Central constriction of a chromosome, separating the short (p) and long (q) arms
2. One of two parallel identical strands of a chromosome, seen during mitosis and meiosis
3. Thread-like packages of genes seen in the nucleus of a cell
4. A specific sequence of DNA that encodes for a protein or polypeptide chain
5. Each of the first 22 pair of chromosomes except the sex chromosomes

Question 91 of 124

A. Trisomy
B. Triploidy
C. Aneuploidy
D. Monosomy
E. Translocation
F. Inversion

Instructions: Match the above options with the correct definition from the choices given below:-

1. A missing chromosome in each cell
2. An extra chromosome in each cell
3. An extra set of the total haploid genome
4. Intra-chromosomal rearrangements
5. Inter-chromosomal rearrangements

Question 92 of 124

A. Autosomal dominant
B. Autosomal recessive
C. X linked recessive
D. Teratogenic
E. Mitochondrial

Instructions: One of the above options best describes the pattern of inheritance of the disorders given below. Please note that the options above can be used for more than one disorder:-

1. Duchenne muscular dystrophy
2. Huntington disease
3. Cystic fibrosis
4. Phenylketonuria
5. Marfan syndrome
6. Fetal alcohol syndrome
7. Leber's hereditary optic neuropathy (LHON

Question 93 of 124

The following statements are correct about autosomal recessive inheritance pattern except:-

A. One or more affected children can be born to unaffected parents
B. There is a higher incidence of consanguinity
C. For carrier parents with one affected child the chance of having another affected baby is 1in 4 (25%)
D. Males and females are affected with equal frequency and severity
E. Male to male transmission is noted

Question 94 of 124

A. 46,XY
B. 47, XXY
C. 45, X
D. 47, XYY
E. 47, XX+ 18
F. 47, XX+13

Instructions: Match the above karyotypes with the correct description from the list given below:-

1. Female with Patau syndrome
2. Female with Edward syndrome
3. Turner syndrome
4. Normal male karyotype
5. Klinefelter syndrome

Question 95 of 124

All the following statements about Turner syndrome are true except:-

A. Dorsal oedema (puffiness of hands and feet) may be the only presenting feature in the newborn period
B. Growth hormone has been successfully used to improve final adult height
C. Infertility is almost universal in Turner syndrome
D. Mental retardation is a common feature
E. Almost 99% of fetuses with 45,X karyotype are spontaneously aborted in early pregnancy

Question 101 of 124

A. Imprinting disorders
B. Uniparental disomy
C. Gonadal mosaicism
D. Mitochondrial inheritance
E. Anticipation
F. Mutation

Instructions: Match the above types of unusual forms of inheritance with the disorders from the list given below:-

1. Huntington disease
2. Severe osteogenesis imperfecta
3. Myoclonic epilepsy with ragged red fibres-MERRF
4. Prader Willi and Angelman syndrome
5. Beckwith Wiedemann syndrome

Question 102 of 124

A. Breast cancer
B. Ovarian cancer
C. Familial adenomatous polyposis
D. Retinoblastoma
E. Lung cancer
F. Wilms tumour

Instructions: Match the above cancers with the known causative genes from the choices given below:-

1. APC gene on Chromosome 5q
2. BRCA1&2
3. RBI gene on Chromosome 13
4. WT gene on Chromosome 11
5. Not usually caused by genes

Question 103 of 124

A. Deformation
B. Disruption
C. Malformation
D. Dysplasia
E. Teratogen
F. Dystocia

Instructions: Match the above options with the best descriptive statement from the choices given below:-

1. Chemical agent with a malign influence on development
2. Abnormal development of a structure caused by extrinsic forces
3. Intrinsic defects in the pattern of development
4. Destruction of normally formed tissue
5. Abnormal organisation of cells in tissue

Question 104 of 124

A. Single field defects
B. Associations
C. Sequence
D. Developmental field complex
E. Synteny
F. Syndrome

Instructions: Match the above options with an example from the list given below:-

1. Hemifacial microsomia
2. Down syndrome
3. CHARGE
4. Potter's syndrome
5. Cleft lip

Question 105 of 124

A. Fetal sexing
B. Ultrasound scanning
C. Chorionic villus sampling
D. Amniocentesis
E. Maternal serum screening
F. Fetal blood sampling

Instructions: Which of the above prenatal diagnostic tests is recommended for the diagnosis of the genetic conditions listed below:-

1. Cystic fibrosis
2. Neural tube defect
3. Down syndrome
4. To check ambiguous karyotype results obtained at amniocentesis
5. X linked disorders with no mutation identified

Question 106 of 124

A raised maternal serum AFP is associated with the all of the following fetal conditions except:-

A. Anencephaly
B. Cleft palate
C. Spina bifida
D. Exomphalos
E. Twins